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Burnouf et al.

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(54) **COMPOUNDS BINDING TO THE BACTERIAL BETA RING**

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C07K 7/06	(2006.01)
C07K 14/245	(2006.01)
C07K 14/195	(2006.01)

(52) **U.S. Cl.**

CPC . ***C07K 7/06*** (2013.01); ***A61K 38/08*** (2013.01); ***C07K 14/195*** (2013.01); ***C07K 14/245*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(74) *Attorney, Agent, or Firm* — Morgan, Lewis & Bockius LLP

(57) **ABSTRACT**

The present invention relates to compounds which bind to the hydrophobic pocket of the β clamp, i.e., to the surface of the β ring with which said protein interacts with other proteins of the bacterial replication complex during DNA replication. These compounds are derived from the acetylated peptide AcQLDLF (P6) to improve their affinity to their target.

16 Claims, 11 Drawing Sheets

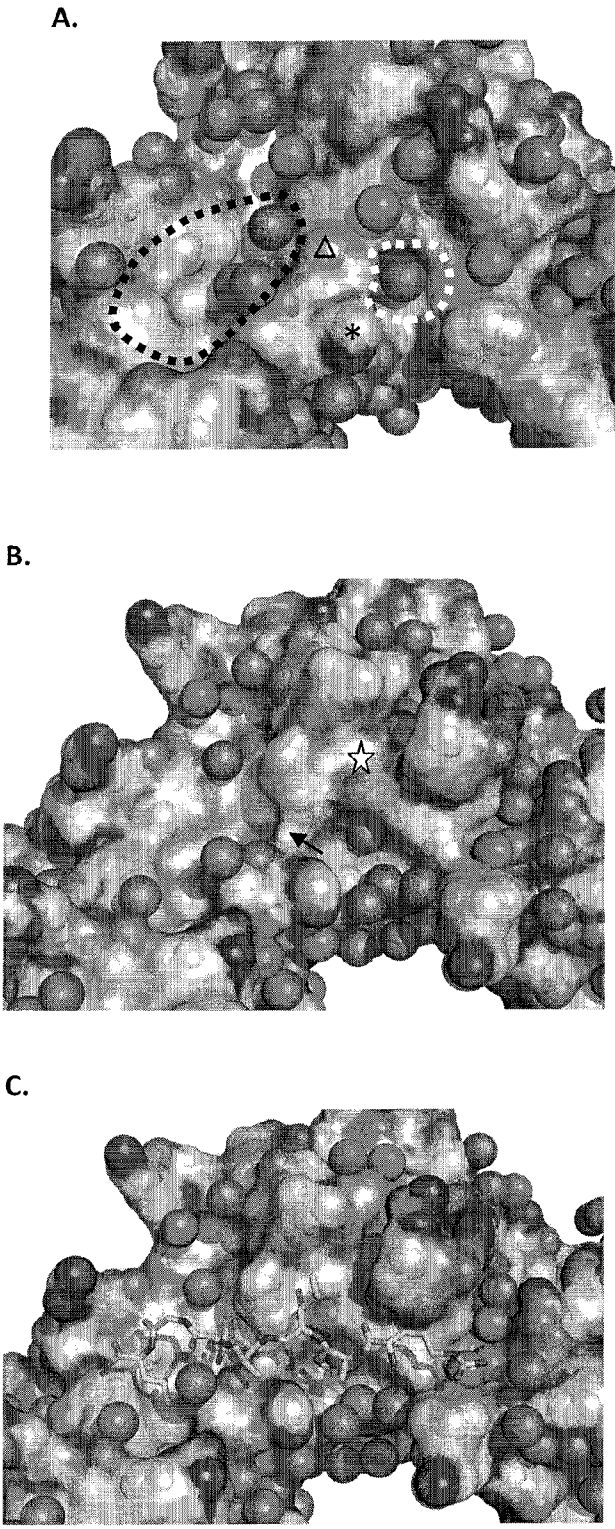


Figure 1

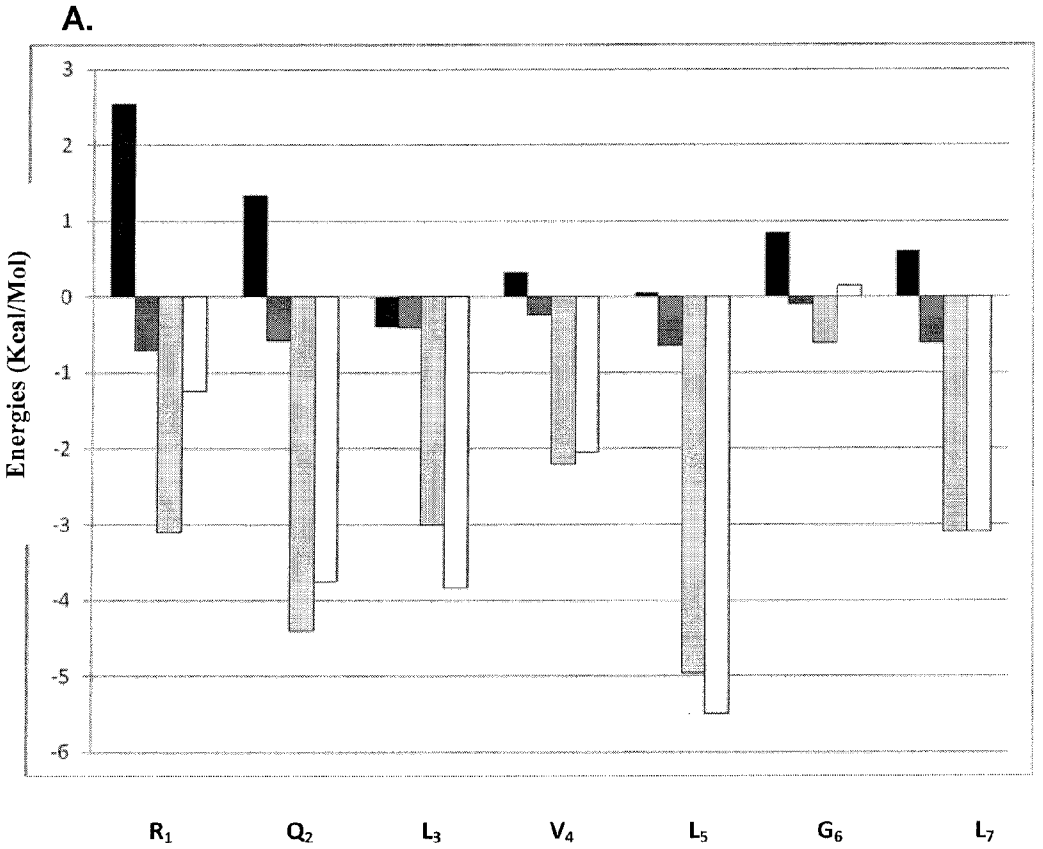
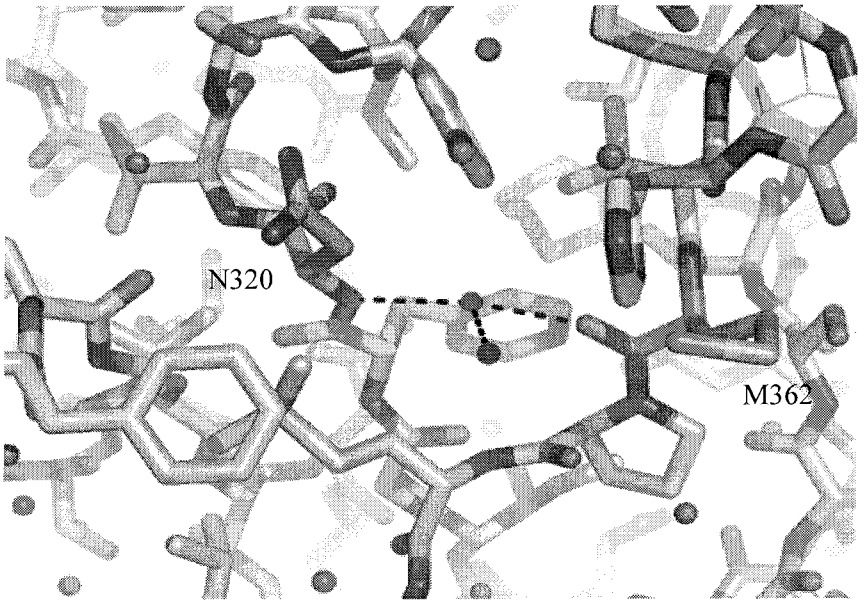

A.
Energies (Kcal/Mol)
3
2
1
0
-1
-2
-3
-4
-5
-6
R1
Q2
L3
V4
L5
G6
L7
B.
Energie (kcal/mol)
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
R1
Q2
L3
V4
L5
G6
L7
Ac-Q1
L2
D3
L4
F5

Figure 2

A.



B.

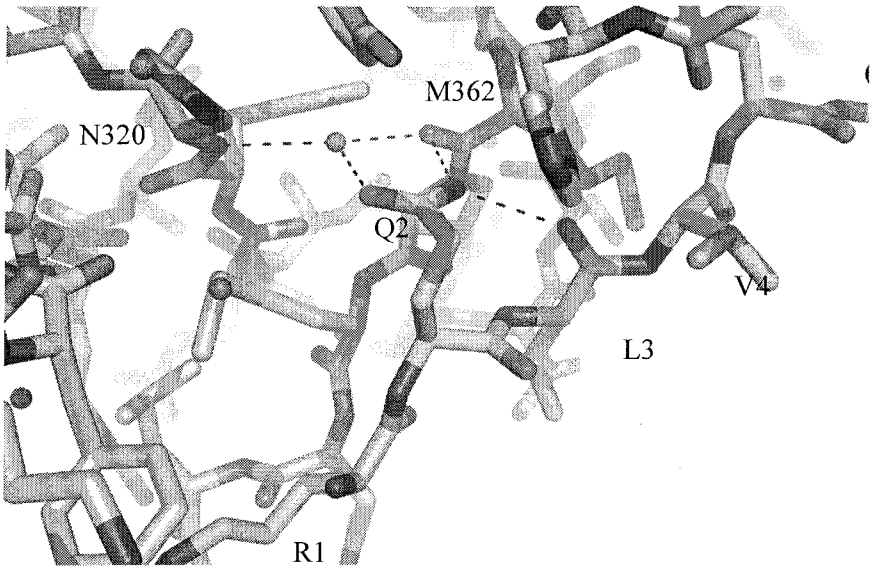
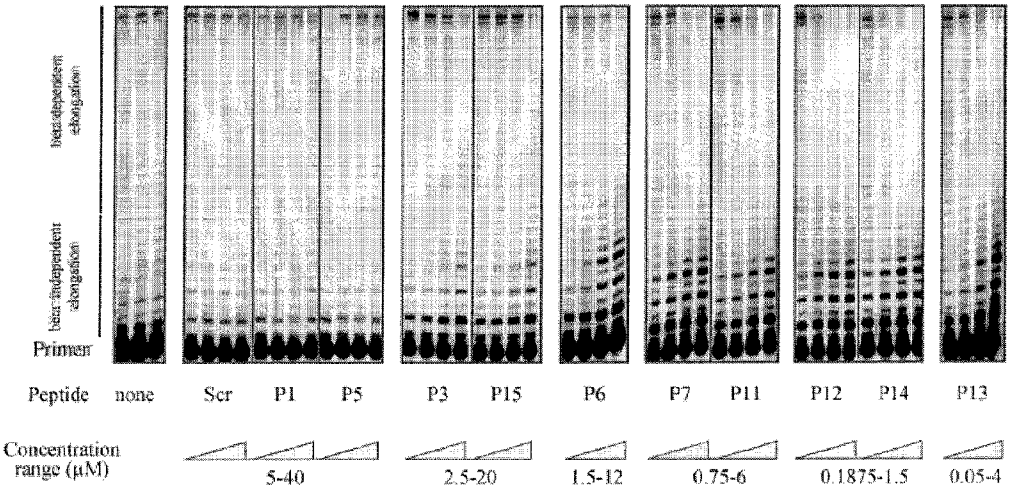


Figure 3

A.



B.

	P1	P5	P3	P15	P6	P7	P11	P12	P14	P13
IC50 Biochemical assay (μM)	nd	nd	7	6,7	4,5	2,8	2	0,3	0,125	0,25
IC50 SPR (μM)	8.85	12.44	8.62	1.91	1.12	0.17	0.26	0.16	0.077	0.096

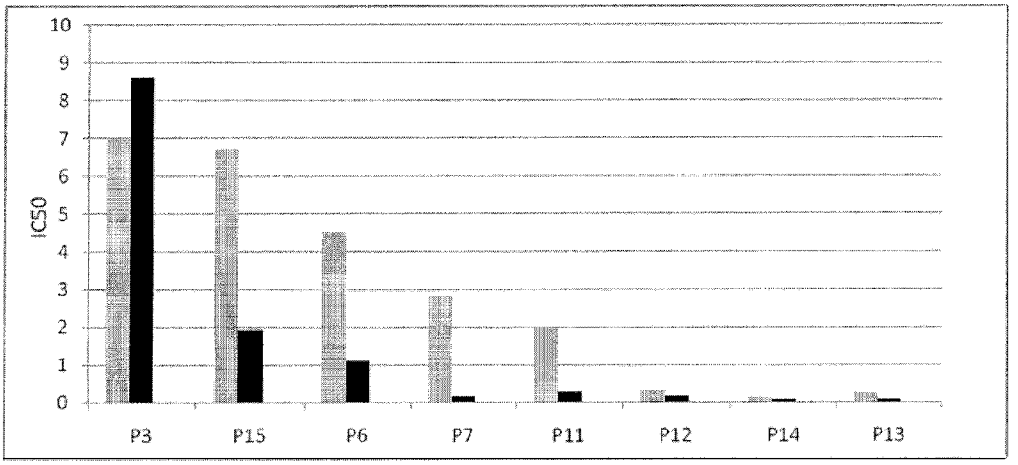


Figure 4

A.

B.

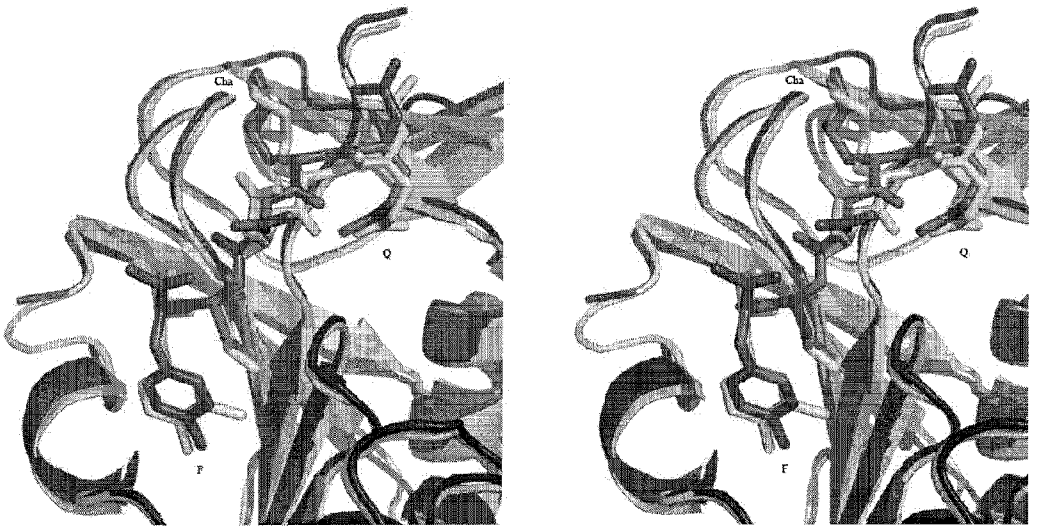


Figure 5

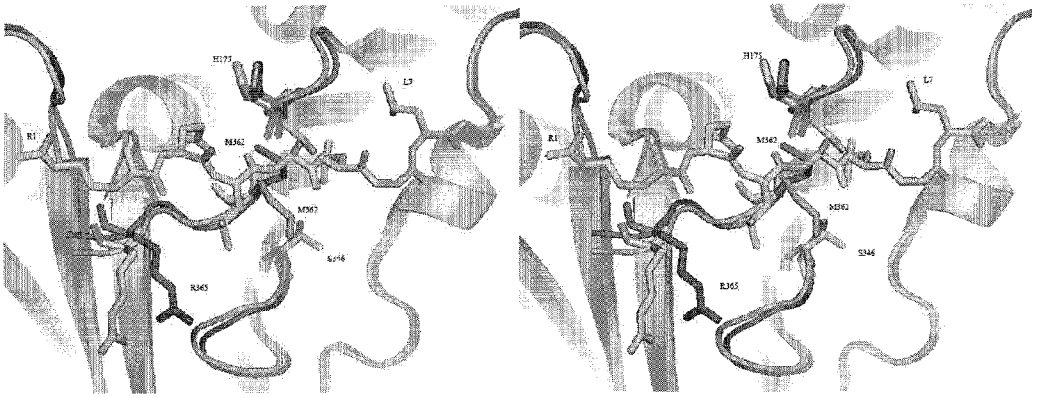


Figure 6

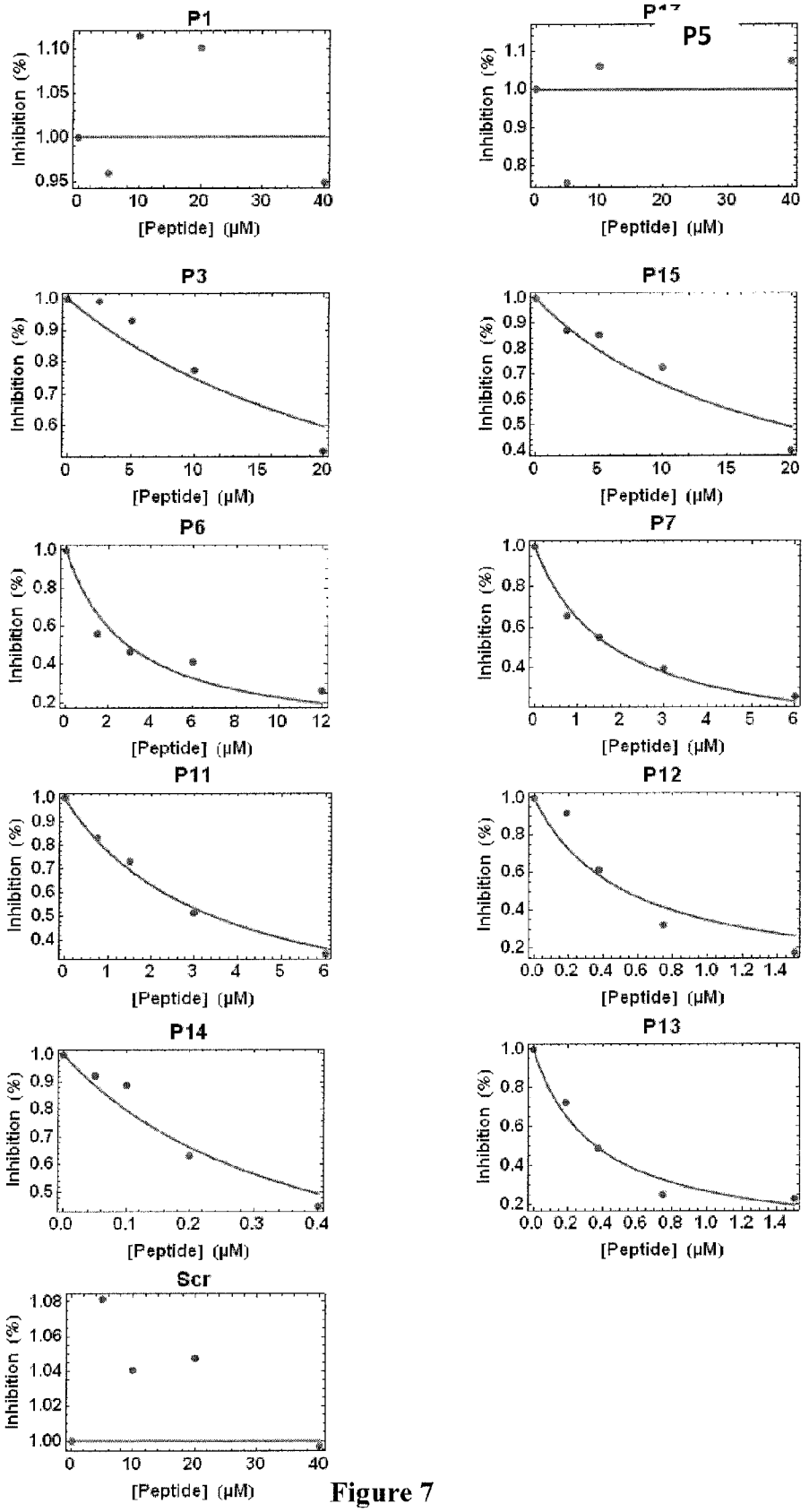
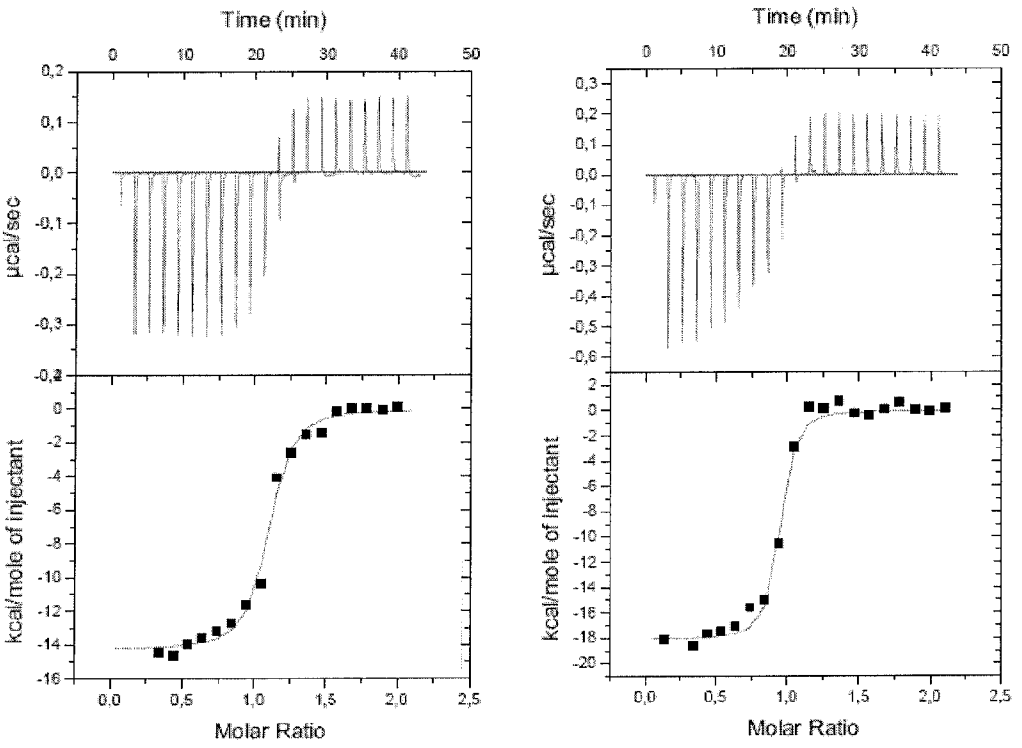


Figure 7

A.



P12, N= 1.06, K_D= 122nM

P14, N= 0.906, K_D= 55nM

B.

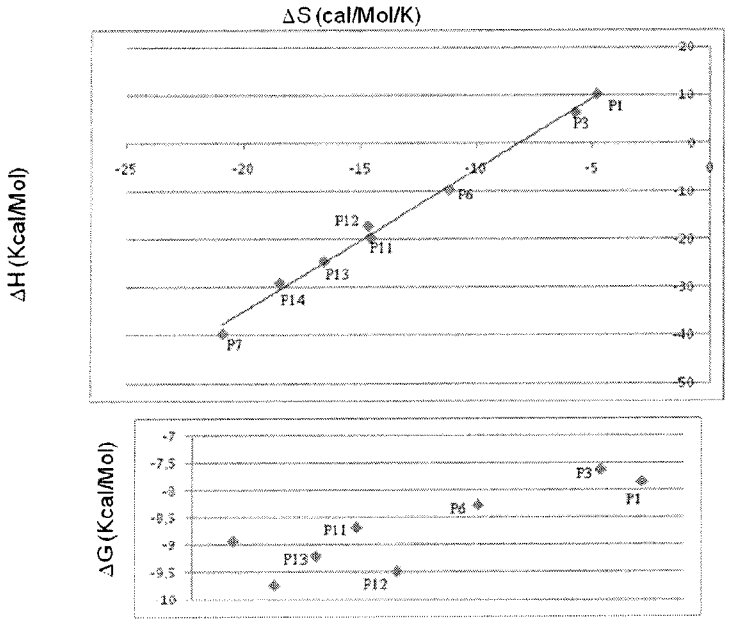
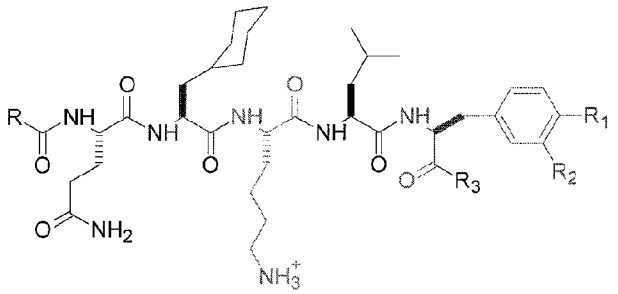
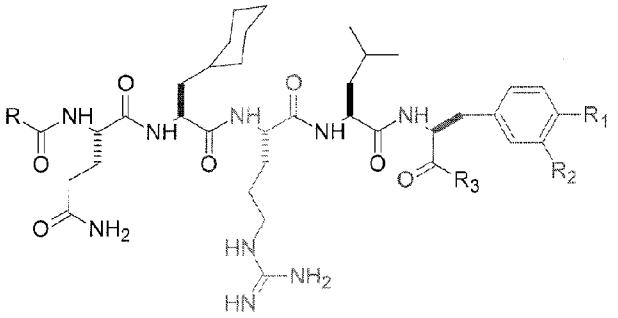


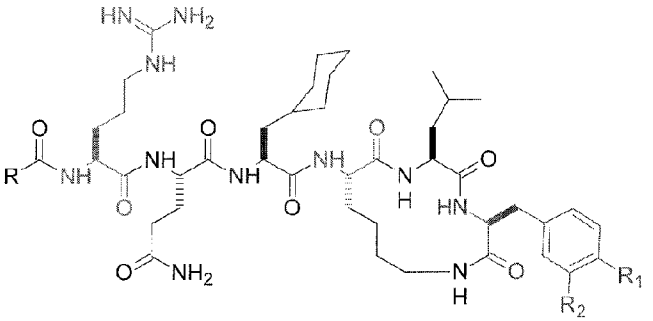
Figure 8



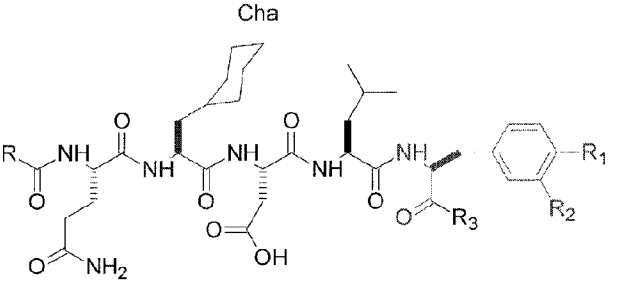
A



B

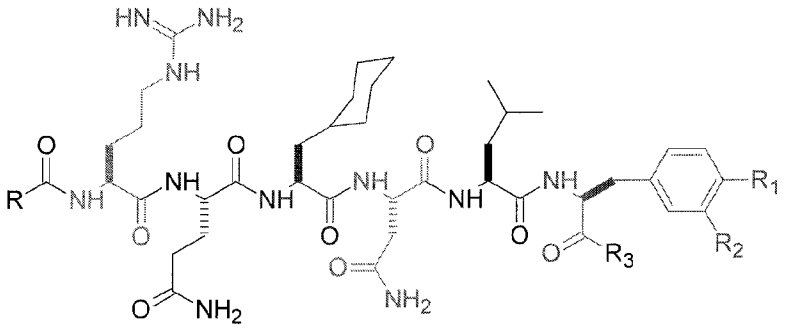


C

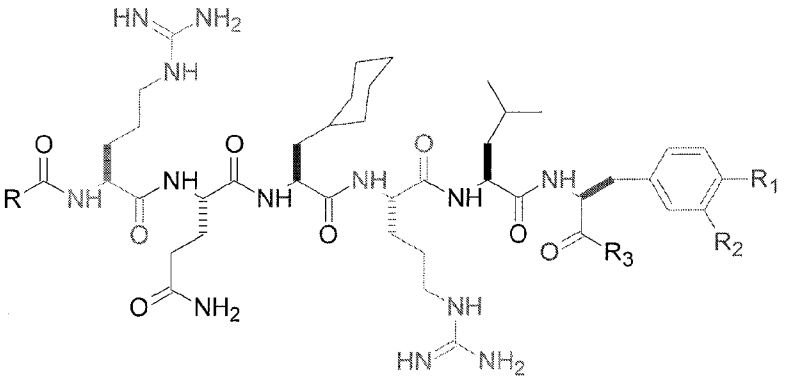


D

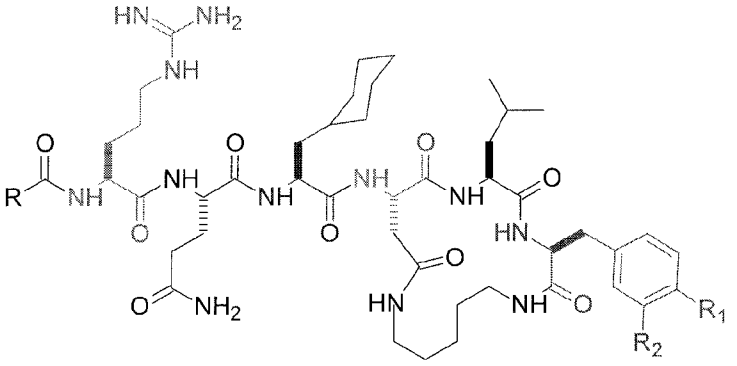
Figure 9 (A-D)



E

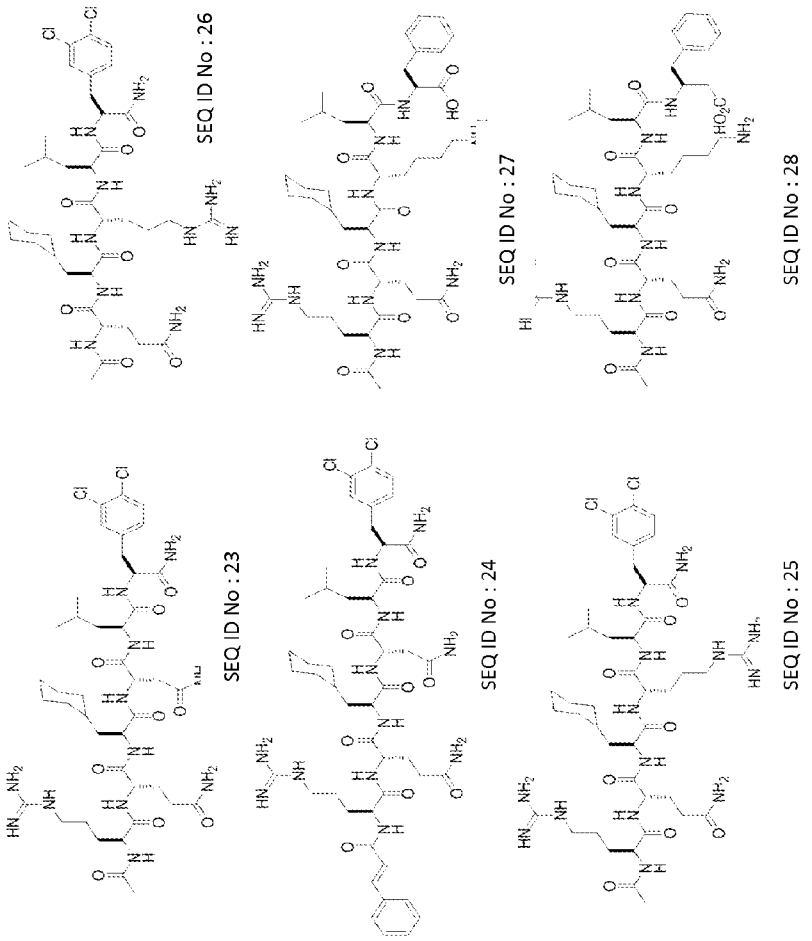


F



G

Figure 9 (E-G)


SEQ ID No : 23
SEQ ID No : 24
SEQ ID No : 25
SEQ ID No : 26
SEQ ID No : 27
SEQ ID No : 28
SEQ ID No : 29
SEQ ID No : 30

H

Figure 9 (H)

COMPOUNDS BINDING TO THE BACTERIAL BETA RING

SEQUENCE LISTING SUBMISSION VIA EFS-WEB

A computer readable text file, entitled "045636-5242-SubstituteSequenceListing.txt" created on or about 14 Apr. 2015, with a file size of about 13 kb contains the sequence listing for this application and is hereby incorporated by reference in its entirety.

The present invention relates to bacterial replication. More precisely, the present invention concerns compounds which bind to the hydrophobic pocket of the β clamp, i.e., to the surface of the β ring with which said protein interacts with other proteins involved in DNA metabolism.

In all three domains of life, multicomponents complexes, the so-called replisomes, have evolved to ensure the faithful replication of chromosomal DNA. One central protein of these complexes forms a ring that encircles and slides along the double stranded DNA[1, 2]. A physical interaction between the clamp and the chromosomal replicase confers a high processivity to the enzyme[3]. In bacteria, the processivity factor, also referred to as the β ring, is a homodimer which results from the head-to-tail association of two monomers, each of them being shaped in three globular sub-domains[1]. In eukaryotes and archae, the β homolog factor, PCNA (for Proliferating Cell Nuclear Antigen), is a homotrimer with each monomer organized in two sub-domains[2, 4].

Beside their role as processivity factors for chromosomal replicases, β and PCNA clamps also participate in various protein-protein interactions. They notably act as landing platforms for factors involved in DNA metabolism and cell cycle regulation[5], particularly DNA polymerases involved in translesion synthesis[6, 7], and factors promoting DNA repair[8, 9, 10]. All these factors possess a small conserved peptide sequence, which binds into a hydrophobic pocket located on one side of the ring. Noteworthy, these pockets differ significantly between bacterial rings and PCNA. A bioinformatics analysis performed on putative β ring partners led to define the bacterial consensus binding peptide QL[S/D]LF[10]. The absolute requirement of the interacting peptide for β ring partners binding has been further demonstrated biochemically and physiologically[11, 12, 13, 14]. Finally, the interaction between the ring and the interacting peptide of different β binding proteins have been structurally characterized[15, 16, 17, 18] The peptide binding site is formed by a deep leucine-rich hydrophobic pocket (subsite 1) located between sub-domains two and three of the β monomer and connected via a groove to a second sub-site (subsite 2) located in sub-domain three[17] (FIG. 1C). An additional interaction has also been observed in the case of the polymerase Pol IV, between the little finger domain of the enzyme and the edge of the β ring[16].

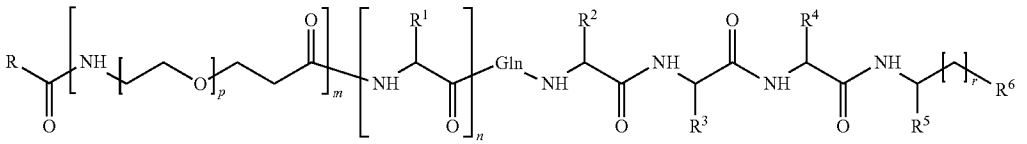
The major contribution of the peptide-mediated interaction to a successful DNA replication and ultimately to cell survival, both in prokaryotes and eukaryotes, makes the ring

interacting pockets potential targets for the development of new antibacterial or anticancer drugs, respectively. In a recent report, a chemical compound was identified from a library and shown to bind into the leucine rich sub-domain of the *E. coli* β ring interacting pocket with an affinity of 10^{-5} M[18].

In the experimental work described below, a different, structure-based strategy was used to design short peptides with improved affinities for the β interacting pocket. The first step of this approach was to decipher the molecular basis of the interaction of the natural ligand in the binding pocket. Then, using these data, a first peptide (SEQ ID No: 6, P6) was designed, which was then further modified to improve its affinity. Several biophysical and biochemical methods were used to measure the strength of the interaction and to characterize the structure of the most efficient complexes formed. As a result, the binding efficiency of the modified ligand was improved by two orders of magnitude, reaching 10^{-8} M range.

Due to their very good affinity for the β interacting pocket, the compounds described in the present text are very promising leads for new antibiotic compounds.

According to a first aspect, the present invention pertains to a compound of formula (I)



(I)

wherein

Gln is glutamine;

R is selected in the group consisting of a C_{1-12}-alkyl group optionally substituted by a C_{6-10}-aryl group, a C_{2-12}-alkenyl group optionally substituted by a C_{6-10}-aryl group, a C_{3-6}-cycloalkyl group, a C_{6-10}-aryl group optionally substituted by a C_{1-4}-alkyl, and a C_{1-5}-alkyl-(O—CH_2—CH_2)$_t$— group with t being an integer from 0 to 20 inclusive;

R^1 is the side chain of arginine or lysine (n.b.: when n>1, each R^1 is, independently from each other, the side chain of arginine or lysine);

R^2 is a —(CH_2)—C_{3-6}-cycloalkyl group optionally substituted by a halogen and/or by a group selected amongst —NH_2, —NH—CO—R^a, —CO_2H, —NHR^a and —N$R^a R^b$, wherein R^a and R^b are independently a C_{1-4}-alkyl group;

R^3 is selected in the group consisting of a C_{1-8}-alkyl group, the side chain of arginine or lysine, —(CH_2)$_q$—CO_2R^{7a}, —(CH_2)$_q$—CO—NHR^{7b}, —CH_2OR^8 and — (CH_2)$_q$ NHR^9, wherein

q is 1, 2, 3 or 4,

R^{7a} is a hydrogen atom, a C_{1-8}-alkyl group, a C_{4-12}-alkylene group forming together with R^6 a lactone or a polyether ring, or a C_{4-12}-alkenylene, forming together with R^6 a lactone or a polyether ring,

R^{7b} is a hydrogen atom, a C_{1-8}-alkyl group, or —(CH_2)$_{q'}$—NH— with q' being an integer between 2 and 8 inclusive and forming together with R^6 a lactam,

R^8 is a hydrogen atom, a C_{1-8}-alkyl group, a C_{4-12}-alkylene group forming together with R^6 a lactone or a polyether ring, or a C_{4-12}-alkenylene, forming together with R^6 a lactone or a polyether ring,

R^9 is a hydrogen atom, or R^9 together with R^6 form a lactam;

R^4 is a C_{1-8}-alkyl group optionally substituted by a C_{3-6}-cycloalkyl group, or a halogen-C_{1-4}-alkyl group;

R^5 is selected in the group consisting of a —(CH_2)—C_{3-6}-cycloalkyl group; —$(CH_2$—$CH_2)$—C_{3-6}-cycloalkyl group; a —(CH_2)—C_{6-10}-aryl group optionally substituted by a halogen, a C_{1-2} alkyl group and/or a C_{1-2} alkoxy group; a —$(CH_2$—$CH_2)$—C_{6-10}-aryl group optionally substituted by a halogen, a C_{1-2} alkyl group and/or a C_{1-2} alkoxy group; a —(CH_2)—C_{5-10}-heteroaryl group optionally substituted by a halogen and/or a C_{1-2} alkyl group; a —$(CH_2$—$CH_2)$—C_{5-10}-heteroaryl group optionally substituted by a halogen and/or a C_{1-2} alkyl group;

R^6 is —CO_2H, —CO_2R^{10}, —CO—NH_2, —CO—NHR^{10}, —OR^{10} when r is 1 or 2, —NH—CO—NHR^{10} when r is 1 or 2, or R^6 is —CO—, —CO—O— or —O— and forms a lactam, a lactone, or a polyether ring with R^{7a}, R^{7b}, R^8 or R^9; wherein

R^{10} is a C_{1-8}-alkyl group optionally substituted by a C_{6-10}-aryl group; a C_{3-6}-cycloalkyl group; a C_{6-10}-aryl group optionally substituted by a halogen, a C_{1-2}-alkyl group and/or a C_{1-2}-alkoxy group;

m is 0 or 1;

n is an integer from 0 to 9 inclusive;

p is an integer from 0 to 10 inclusive;

r is 0, 1 or 2.

In the above formula (I), the peptide linkages (—CO—NH—) can be replaced or modified to obtain synthetic pseudopeptides or peptidomimetics in which the peptide bond is modified, especially to become more resistant to proteolysis, provided the immunogenicity of and the toxicity of the molecule is not increased by this modification, and providing the pseudopeptide retains its affinity for the β interacting pocket.

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein. The term "C_{1-12}-alkyl" refers to a branched or straight-chain monovalent saturated aliphatic hydrocarbon group of 1 to 12 (inclusive) carbon atoms. Similarly, the terms: "C_{1-8}-alkyl", "C_{1-5}-alkyl", "C_{1-4}-alkyl", "C_{1-2}-alkyl" and the like refer to branched or straight-chain monovalent saturated aliphatic hydrocarbon groups of, respectively, 1 to 8 (inclusive), 1 to 5 (inclusive), 1 to 4 (inclusive), 1 to 2 carbon atoms. This term is further exemplified by groups as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecanyl and their branched isomers. The "alkyl" group can optionally be mono-, di-, tri- or multiply-substituted by a halogen and/or a C_{6-10} aryl group, as defined below.

The term "C_{1-8}-alkyl-(O—CH_2—$CH_2)_t$-" refers to a —(O—CH_2—$CH_2)_t$— substituted C_{1-8}-alkyl group wherein the alkyl group is as defined above and t is an integer from 0 to 20 (inclusive), preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Preferred —(O—CH_2—$CH_2)_t$— substituted alkyl group is a C_{1-5}-alkyl-(O—CH_2—$CH_2)_t$— group with t and alkyl as defined above.

The term "C_{2-12}-alkenyl" refers to a branched or straight-chain monovalent unsaturated aliphatic hydrocarbon group having one or more carbon double bonds, of 2 to 12 (inclusive) carbon atoms, preferably 2 to 8 (inclusive) carbon atoms, more preferably 2 to 4 (inclusive) carbon atoms. This term is further exemplified by groups as vinyl, propylenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl, undecenyl, dodecenyl and their straight-chain and branched and stereo isomers. The "alkenyl" group can optionally be mono-, di-, tri- or multiply-substituted by a halogen and/or a C_{6-10}-aryl group, as defined below.

The term "C_{1-12}-alkylene" refers to a divalent C_{1-12}-alkyle with alkyl as defined above. Similarly, terms such as "C_{4-12}-alkylene" or "C_{4-8}-alkylene" and the like, refer to divalent C_{4-12}-alkyl or divalent C_{4-8}-alkyle group where alkyl is defined above. Examples of alkylene groups are —(CH_2)—, —$(CH_2)_2$—, —$(CH_2)_3$—, —$(CH_2)_4$—, —$(CH_2)_5$—, —$(CH_2)_6$—, —$(CH_2)_7$—, —$(CH_2)_8$—, —$(CH_2)_9$—, —$(CH_2)_{10}$—, —$(CH_2)_{11}$—, —$(CH_2)_{12}$—.

The term "C_{4-12}-alkenylene" refers to a divalent C_{4-12}-alkenyl of formula —$(CH_2)_x$—$(CH$═$CH)_y$—$(CH_2)_z$— wherein x and z are, independently, 0, 1, 2, 3, 4, 5, 6, 7 or 8 and y is 1, 2, 3 or 4. Similarly, the term "C_{4-8}-alkenylene", refers to a divalent C_{4-8}-alkenyl. Examples of alkenylene groups include butenyl, pentenyl, pentadienyl, hexenyl, hexadienyl, heptenyl, heptadienyl, octenyl, octadienyl, nonenyl, nonadienyl, decenyl, decadienyl, undecenyl, undecadienyl, undodecenyl, undodecadienyl, and their straight-chain and branched and stereo-isomers.

The term "C_{3-6}-cycloalkyl" refers to a saturated or partially unsaturated cyclic hydrocarbon group having 3 to 6 (inclusive) carbon atoms. This term is further exemplified by groups as cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. The "C_{3-6}-cycloalkyl" group can optionally be mono-, di-, tri- or multiply-substituted by a halogen as defined below, a C_{1-4}-alkyl group as defined above, a —NH_2, a —NH—CO_2H, a —NH—CO—R^a, —CO_2H, —NHR^a and/or —NR^aR^b wherein R^a and R^b are independently a C_{1-4}-alkyl group as defined above.

The term —(CH_2)—C_{3-6}-cycloalkyl group refers to a —CH_2— substituted C_{3-6}-cycloalkyl group wherein the cycloalkyl group is as defined above.

The term "C_{6-10} aryl" refers to a monocyclic or bicyclic aromatic ring system of 6 to 10 (inclusive) carbon atoms, preferably 6 carbon atoms. This term is further exemplified by groups as phenyl and naphtyl. The C_{6-10}-aryl group can optionally be mono-, di-, tri- or multiply-substituted by a halogen as defined below and/or a C_{1-4}-alkyl group as defined above.

The terms "halo" or "halogen" refers to fluorine, chlorine, bromine and iodine.

The term "halogen-C_{1-4}-alkyl", refers to a halogen substituted C_{1-4}-alkyl group wherein both halogen and alkyl groups have the meaning as above. Preferred "halogen-C_{1-4}-alkyl" groups are fluorinated "halogen-C_{1-4}-alkyl" groups such as —CF_3, —CH_2—CF_3, —$CH(CF_3)_2$, —$CH(CH_3)(CF_3)$, —C_4F_9.

The term "C_{1-12}-alkoxy" refers to a branched or straight-chain monovalent saturated aliphatic hydrocarbon group of 1 to 12 (inclusive) carbon atoms attached to an oxygen atom. Similarly, the terms "C_{1-8}-alkoxy", "C_{1-5}-alkoxy", "C_{1-4}-alkoxy", "C_{1-2}-alkoxy" refer to branched or straight-chain monovalent saturated aliphatic hydrocarbon groups of, respectively, 1 to 8 (inclusive), 1 to 5 (inclusive), 1 to 4 (inclusive), 1 to 2 carbon atoms. Examples of "alkoxy" groups are methoxy, ethoxy, propoxy, butoxy, pentoxy, hexyloxy, heptyloxy, octyloxy, and their branched isomers.

The term "C_{5-10}-heteroaryl" refers to a heterocyclic aryl group containing 1 to 3 heteroatoms in the ring with the remainder being carbon atoms. In the said heterocyclic aryl group, suitable heteroatoms include, without limitation, sulfur and nitrogen. Exemplary heteroaryl groups include indolyl, azaindolyl, thiophenyl, benzothiophenyl, thioazolyl, benzothiazolyl. The heteroaryl group can optionally be mono-, di-, tri- or multiply-substituted by a halogen and/or a C_{1-4}-alkyl group, as defined above. When the heteroaryl

group is mono-, di-, tri- or multiply-substituted by a C_{1-4}-alkyl group, said alkyl group is preferably a methyl group.

The term "polyether ring", refers ring containing 1, 2, or 3 ether groups, an ether group being an oxygen atom connected to two alkyl groups as defined above

The term "lactone" refers to a closed ring containing an oxygen atom adjacent to a carbonyl group (—CO—O—). It can be considered as the condensation product of an —OH group with a $—CO_2H$ group.

The term "lactam" refers to a closed ring containing an nitrogen atom adjacent to a carbonyl group (—CO—NH— or —CO—NR— with R being for example an alkyl group as defined above).

The terms "substituted" and "substitution and the like", refer to the replacement of one, two, three or more atoms in a given group by one, two, three or more suitable substituents, including, without limitation, a halogen, a C_{6-10} aryl group, a C_{1-4}-alkyl group, a C_{1-2}-alkyl group, a C_{1-2}-alkoxy group, a $—NH_2$, a —NH—CO—R^a, $—CO_2H$, —NHR^a and/or —N$R^a R^b$ wherein R^a and R^b are independently a C_{1-4}-alkyl group, or a mixture of those substituents.

In some embodiments of the invention, the compounds of the invention can contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereoisomeric mixtures. All such isomeric forms of these compounds are included in the present invention, unless expressly provided otherwise.

In some embodiments, the compounds of the invention can contain one or more double bonds and thus occur as individual or mixtures of Z and/or E isomers. All such isomeric forms of these compounds are included in the present invention, unless expressly provided otherwise.

In the embodiments where the compounds of the invention can contain multiple tautomeric forms, the present invention also includes all tautomeric forms of said compounds unless expressly provided otherwise.

In the embodiment where R^{7a} together with R^6 form a lactone or a polyether ring,

- R^{7a} is C_{4-12}-alkylene, preferably C_{4-8}-alkylene group, and is linked to a —CO—O— or to a —O— functional group in R^6, or
- R^{7a} is C_{4-12}-alkenylene, preferably C_{4-8}-alkenylene group, and is linked to a —CO—O— or to a —O— functional group in R^6.

In the embodiment where R^{7b} together with R^6 form a lactam, R^3 is a $—(CH_2)_q—CO—NHR^{7b}$ and R^{7b} is $—(CH_2)_{q'}$—NH— with q' being 2, 3, 4, 5, 6, 7 or 8.

In the embodiment where R^9 together with R^6 form a lactam, R^3 is a $—(CH_2)_qNHR^9$ and R^9 is a direct link between $—(CH_2)_qNH—$ and a —CO— functional group in R^6.

In the embodiment where R^8 together with R^6 form a lactone or a polyether ring:

- R^8 is C_{4-12}-alkylene, preferably C_{4-8}-alkylene group, and is linked to a —CO—O— or to a —O— functional group in R^6, or
- R^8 is C_{4-12}-alkenylene, preferably C_{4-8}-alkenylene group, and is linked to a —CO—O— or to a —O— functional group in R^6.

The terms "β ring", "β protein" or "β clamp" herein designate the β subunit of a eubacterial DNA polymerase III, such as that of *E. coli*. The β subunit of DNA polymerase III of *E. coli* is in particular described in Kong et al. (1992)[1].

Further definitions are added in the text, when necessary.

Particular embodiments of the compounds according to the invention are described in the following more detailed specification.

According to a particular embodiment of the compounds according to the invention, the R group indicated in the above formula (I) is selected amongst a C_{1-8}-alkyl group optionally substituted by a C_{6-10}-aryl group, a C_{2-8}-alkenyl group optionally substituted by a C_{6-10}-aryl group or a C_{1-5}-alkyl-$(O—CH_2—CH_2)_t—$ group with t being 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Preferably, R is a C_{1-4}-alkyl group optionally substituted by a C_{6-10}-aryl group or a C_{2-4}-alkenyl group optionally substituted by a C_{6-10}-aryl group. Indeed, as described in the experimental part below, the inventors have observed that a N-terminal acetylation of the peptide P5 (QLDLF, SEQ ID No: 5) leads to a 10-fold increase of the affinity of the peptide for the β interacting pocket.

When m is not null and p is >1 in the above formula (I), for example when m=1 and p=2, 3, 4, 5, 6, 7, 8, 9 or 10, the above-described compounds are pegylated. The pegylation contributes to the stability of the whole molecule and can also have a positive effect for the entry of said compound into bacterial cells.

Particular compounds according to the invention have one or several arginines and/or lysines at the N-terminal extremity of the peptide part of the compound. For example, n is 1, 2, 3, 4 or 5. Indeed, these positively charged residues are known to favor membrane crossing. This feature is however not compulsory, and other compounds according to the invention do not have such residues (n=0 in formula I).

The inventors have also observed that substitution of the second amino acid of Ac-QLDLF (SEQ ID No: 6, P6) by a beta-cyclohexyl-L-alanyl (hereafter designated as "Cha") leads to a further 6-fold increase of the affinity of said peptide with the β hydrophobic pocket. Other substitutions at the same position (see Table 4 in the experimental part) led to the above definition of R^2 in formula (I). According to an advantageous embodiment, R^2 is a $—(CH_2)—C_{3-6}$-cycloalkyl group.

By substituting the leucine of the acetylated peptide Ac-Gln-Cha-Asp-Leu-Phe 7 (SEQ ID No: 7, P7) by a number of different residues (see Table 5 of the experimental part below), the inventors could determine preferable embodiments for R^4 group, in order to optimize the binding to the interacting pocket of the β ring. Accordingly, R^4 is preferably selected amongst C_{1-5}-alkyl groups, especially branched ones (such as valine, leucine or homoleucine lateral chains, for example), or amongst $C_{1-3}—$, preferably C_{1-2}-alkyl groups optionally substituted by a C_{3-6}-cycloalkyl group (such as Cha and homoCha, for example).

As shown in Table 6 below, modifications introduced on the C-terminal phenylalanine benzyl ring led to an increase of the affinity of the molecule with the interacting pocket of the β ring. The binding affinity was found to increase with the size of the ring substituent (p-methyl<p-chloro<p-bromo<3,4-dichloro). The same table shows that other cyclic molecules can be used in this position. Contrarily, replacement of the phenylalanine lateral chain by a 2-amino-tetradecanoic acid led to a significant loss in affinity, thereby indicating an upper limit for the size of the group to be used at this position. Accordingly, in the compounds of the present invention, R^5 is preferably a $—(CH_2)—C_{6-10}$-aryl group optionally substituted by a halogen, a C_{1-2} alkyl group and/or a C_{1-2} alkoxy group.

As exemplified in the experimental part below, excellent affinities are obtained with linear molecules having a peptidic skeleton. Such molecules excellently mimic the binding part of the proteins which naturally interact with the β ring. When the compounds according to the invention are linear, R^3 and R^6 are as follows:

R^3 is selected in the group consisting of a C_{1-8}-alkyl group, the side chain of arginine or lysine, $—(CH_2)_q—CO_2R^{7a}$, $—(CH_2)_q—CO—NHR^{7b}$, $—CH_2OR^8$, $—(CH_2)_qNHR^9$, wherein

q is 1, 2, 3, 4,

R^{7a} is a hydrogen atom, or a C_{1-8}-alkyl group,

R^{7b} is a hydrogen atom, or a C_{1-8}-alkyl group,

R^8 is a hydrogen atom, a C_{1-8}-alkyl group,

R^9 is a hydrogen atom;

and

R^6 is $—CO_2H$, $—CO_2R^{10}$, $—CO—NH_2$, $—CO—NHR^{10}$, $—OR^{10}$ when r is 1 or 2, $—NH—CO—NHR^{10}$ when r is 1 or 2; wherein

R^{10} is a C_{1-8}-alkyl group optionally substituted by a C_{6-10}-aryl group; a C_{3-6}-cycloalkyl group; a C_{6-10}-aryl group optionally substituted by a halogen, a C_{1-2}-alkyl group and/or a C_{1-2}-alkoxy group.

It is to be noted that R^6 is directed towards the solvent. Hence, R^6 can be replaced by virtually any kind of molecule. For example, it can advantageously be replaced by or attached to a molecule which helps the crossing of membranes and/or the internalization by the bacteria. Non-limitative examples of such molecules are cell-penetrating peptides (CPP) (Classes and prediction of cell-penetrating peptides, Lindgren M, Langel U., Methods Mol Biol. 2011, 683, p. 3-19). In case a CPP or another molecule is covalently bound to the compound via R^6, a linker, made of one to 10, preferably 1 to 4 amino acids, can be added between the compound of the invention and said CPP. Such a linker can be, for example, a mere arginine or lysine, or a sequence of 2 to 4 amino-acids corresponding to the amino-acids immediately following the binding site of a natural ligand of the β ring, such as, for example, ASRQ (SEQ ID No: 31), which is the sequence following the binding site of the delta protein from the gamma complex. Indeed, as shown by Jeruzalmi et al (2001)[15, 15a], this protein exhibits a bend towards the outside of the pocket. Hence, a CPP bound via a ASRQ linker to a compound according to the invention would not hinder the interaction of said compound with the β ring.

According to a particular embodiment of the linear compounds of the present invention, R^3 and/or R^6 are as follows:

R^3 is selected in the group consisting of the side chain of arginine, the side chain of lysine, $—(CH_2)_q—CO_2R^{7a}$ and $—(CH_2)_q—CO—NHR^{7b}$, wherein

q is 1, 2, 3 or 4,

R^{7a} is a hydrogen atom, or a C_{1-8}-alkyl group, and

R^{7b} is a hydrogen atom, or a C_{1-8}-alkyl group,

and/or

R^6 is $—CO_2H$ or $—CO—NH_2$.

In an alternative embodiment, the compounds according to the present invention are cyclic, a cycle being made between the R^3 and R^6 groups. This bridge between R^3 and R^6 groups eliminates carboxylates, thereby improving the capacity of the compounds to enter bacterial cells, without impacting R5, which is necessary for anchoring the compound in the pocket, and for the subsequent conformational modification of said pocket. According to this embodiment, R^3 and R^6 are as follows:

R^3 is selected in the group consisting of $—(CH_2)_q—CO_2R^{7a}$, $—(CH_2)_q—CO—NHR^{7b}$, $—CH_2OR^8$, $—(CH_2)_qNHR^9$, wherein

q is 1, 2, 3 or 4,

R^{7a} is a C_{4-8}-alkylene group forming together with R^6 a lactone or a polyether ring, or a C_{4-8}-alkenylene, forming together with R^6 a lactone or a polyether ring,

R^{7b} is $—(CH_2)_{q'}—NH—$ with q' being an integer from 2 to 8 inclusive and forming together with R^6 a lactam,

R^8 is a C_{4-8}-alkylene group forming together with R^6 a lactone or a polyether ring, or a C_{4-8}-alkenylene, forming together with R^6 a lactone or a polyether ring,

R^9 together with R^6 form a lactam;

R^6 is —CO—, —CO—O— or —O— and forms a lactam, a lactone, or a polyether ring with R^{7a}, R^{7b}, R^8 or R^9.

Particular compounds according to the present invention are described in the experimental part which follows. Particular compounds having a very good to excellent affinity for the β ring are: P7 (SEQ ID No: 7), P11 (SEQ ID No: 11), P12 (SEQ ID No: 12), P13 (SEQ ID No: 13), P14 (SEQ ID No: 14), P16 (SEQ ID No: 16), P17 (SEQ ID No: 17), P23 (SEQ ID No: 23), P24 (SEQ ID No: 24), P25 (SEQ ID No: 25), P26 (SEQ ID No: 26), P27 (SEQ ID No: 27).

As described in the experimental part below and as perfectly known by skilled artisans, several techniques exist to measure the affinity of two interacting proteins. These techniques may give slightly different results. However, the relative affinity of two compounds for the β ring is not dependent from the technique used for measuring said affinities (FIG. **4**B). In a preferred embodiment of the compounds according to the invention, the affinity of said compounds for the interacting pocket of the bacterial β ring is at least twice the affinity of the acetylated peptide of sequence AcQLDLF (SEQ ID No: 6, P6) with said interacting pocket.

The compounds described above can advantageously be used as antibacterial agents, since they inhibit, at least partially, the interaction between the β protein and proteins that interact therewith by binding to its hydrophobic pocket.

A pharmaceutical composition comprising, as an active agent, a compound as above-described, is also part of the present invention.

FIGURES LEGENDS

FIG. **1**: Representations of the ligand binding pocket of the β ring of *E. coli*, from the co-crystal structure of the β ring with the C-terminal peptide of the *E. coli* DNA polymerase IV ($R_1Q_2L_3V_4L_5G_6L_7$, SEQ ID No: 32) (PDB code 1OK7). A: unbound pocket: the M_{362} (Δ) residue is located close to the H_{175} β residue (*) and obstructs the path between subsite 1 (black dots area) and subsite 2 (white dots area). Water molecules are represented as medium grey balls. B: bound pocket. The peptide has been removed. The movement of residue M_{362} opens a cleft (dark arrow) which connects subsite 1 and subsite 2 and where the V_4 peptide residue interacts (see C). Water molecules are displaced, as compared to A, so that the peptide can fit into subsite 1. Note the opening of the platform (white star) between M_{362} and R_{365} where the L_3 peptide residue will be located. C: Same as B but with the peptide P1 bound into the pocket.

FIG. **2**: A: Energetic contributions (Kcal/mol) of each peptide residue ($R_1Q_2L_3V_4L_5G_6L_7$, SEQ ID No: 32) for the interaction within the binding pocket of the β ring (PDB 1OK7). Black: electrostatic contribution, dark grey: solvent accessible surface contribution, light grey: Van der Waals contribution, white: total contribution. B: Single residue contribution (kcal/mol) to the peptide binding. Native peptide P1 of *E. coli* DNA polymerase IV, from the structure 1OK7, is in black. The pentapeptide P6 is in grey (PDB 3Q4J).

FIG. **3**: Detailed connectivities between β residues N_{320} and M_{362} in subsite 2 of the binding pocket, in absence (A) or presence (B) of the peptide. Balls represent water molecules. From PDB structure 1OK7, incorporated herein by reference.

FIG. **4**: Polymerase competition assay. A: the β dependant activity of PolIV DNA polymerase is challenged by increasing concentrations of various peptides B: the table displays the IC_{50} determined for various peptides by the Pol IV based biochemical assay and the SPR assay. The histogram indicates that the same general trend is observed with both techniques despite a difference in sensitivity. Grey: biochemical assay, black: SPR assay. P15 sequence is Ac-RQLVLF, (SEQ ID No: 15), Scr: scrambled peptide: Ac-ChaFQLD, (SEQ ID No: 33).

FIG. **5**: Superimposition of peptide-β complexes. A: A P6-β complex (pale colors) is superimposed on P12-β complex (dark colors) (rmsd: 0.95 Å). The first (Gln) and last (Phe) peptide residues are indicated. The Cha group of P12 (SEQ ID No: 12) peptide occupies the same position as the Leu_2 residue of P6 (SEQ ID No: 6). The chloro-modified Phe residue of P12 is tilted toward the bottom of subsite 1 as compared to the cognate residue of P6. B: P14-β complex (pale colors) is superimposed on P12-β complex (dark colors) (rmsd: 0.56 Å). The chlorine atom in meta position forms an halogen bond with T172 residue.

FIG. **6**: Superposition of the peptide free (dark) and peptide bound (pale) interacting pockets of 1OK7 structure. In the absence of peptide, the M_{362} side chain (dark) is located close to the H_{175} residue (closed conformation), and separates subsite 1 and subsite 2. When the peptide is bound, the M_{362} side chain (pale) is displaced away from the H_{175} (open conformation) allowing the opening of a cleft in which the peptide can bind. Residue R_{365} is also shifted upon peptide binding, triggering the opening of a small platform where the peptide L_3 residue locates.

FIG. **7**: Graphical representation of the quantitative analysis of polymerase competition assays performed with several peptides. The percentage of inhibition of β dependent *E. coli* DNA polymerase IV activity is plotted as a function of peptide concentration (μM). P15 sequence is Ac-RQLVLF (SEQ ID No: 15). Scr: scramble peptide: Ac-ChaFQLD (SEQ ID No: 33); (related to FIG. **4**).

FIG. **8**: Isothermal titration calorimetry (ITC).

A. Binding isotherms for the titration of the β ring with peptide P12 (SEQ ID No: 12) and P14 (SEQ ID No: 14). N: number of sites per β monomer.

B. Enthalpy-entropy compensation for selected natural and non-natural β binding peptides. The thermodynamics parameters are determined by ITC. Each value is the mean of two independent experiments monitoring the binding of each peptide (400 μM) to the β ring (20 or 30 μM) at 25° C. Each correlation point is labeled according to the corresponding peptide, and the respective AG values are plotted below. 1 cal=4.18J; (related to Table 11).

FIG. **9**: Examples of compounds according to the invention are represented in FIG. **9** (A-H). Those include compounds wherein R=acetyl, cynamoyle, octanoyle; R^1=Cl and R^2=H, or R^1=R^2=Cl, or R^1=R^2=H, or R^1=Me and R^2=H, or R^1=Br and R^2=H; and R^3=OH or NH_2. Specific compounds P23 (SEQ ID No: 23), P24 (SEQ ID No: 24), P25 (SEQ ID No: 25), P26 (SEQ ID No: 26), P27 (SEQ ID No: 27), P28 (SEQ ID No: 28), P29 (SEQ ID No: 29) and P30 (SEQ ID No: 30) are disclosed in FIG. **9**H.

EXAMPLES

Example 1

Structure-Based Design of Short Peptide Ligands Binding onto the *E. coli* Processivity Ring

1.1. Material and Methods

1.1.1. Protein Production, Purification and Characterization

The *E. coli* dnaN gene was cloned into pET15b plasmid (Invitrogen) using standard protocols. The resulting N-tagged protein was expressed in BL21 *E. coli* cells after IPTG induction (0.1 mM) at 28° C. The β protein fraction was first enriched on a Ni-NTA column, eluted with an histidine step (300 mM) and further purified on a MonoQ column in buffer containing 20 mM Tris HCl pH 7.5, 0.5 mM EDTA and 10% glycerol, using a gradient from 0 to 0.5 M NaCl. The quality of the protein was assessed by mass spectrometry in denaturing and native conditions

1.1.2. Peptide Synthesis

Peptides P1-P14 (SEQ ID Nos: 1 to 14) were synthesized in Fmoc chemistry by the stepwise solid-phase methodology[28] on a home-made semi-automatic peptide synthesizer[29]. N—N-Fmoc protected amino acids (natural and non natural) are commercially available from Polypeptide Labs (Strasbourg, France). Resins for solid-phase peptide synthesis are commercially available from Polypeptide Labs (Strasbourg, France) and CBL Patras (Patras, Greece). Assembly of the protected peptide chains was carried out on a 100-μmol scale starting from either Fmoc-Leu-Wang resin (Peptides P1, P2, P4), Fmoc-Phe-Wang (Peptides P3, P5-P10) resin or o-chlorotrityl chloride resin (peptide P11-P14). For each coupling step, the reactants were introduced manually as a solution in dry DMF (2.0 mL). Nα-Fmoc amino acids (5.0 equivalent) with standard side-chain protecting groups were coupled 2 times by using BOP (5.0 equivalent), HOBt (5.0 equivalent) and DIEA (10.0 equiv) in dry DMF for 20 min. The washing of the resin as well as Fmoc deprotection (by using a freshly prepared solution of 20% piperidine in DMF) were performed automatically. The coupling and deprotection steps were monitored by the Kaiser test[30]. At the end of the elongation of the peptidic chain, the resin was washed with CH_2Cl_2 and dried with Et_2O. A mixture of TFA/H_2O/TIPS/DTT (8.8/0.5/0.2/0.5; 10.0 mL) was then added to the resin. The mixture was gently shaken for 2.5 h and the resulting solution was flushed through a frit in cold Et_2O. The precipitate was recovered by centrifugation, dissolved in a mixture of AcOH and H_2O and freeze-dried. The crude peptides were finally purified by HPLC (linear gradient, 5-65% B, 30 min) and freeze-dried. All peptides were identified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), and their homogeneity was assessed by C_{18} RP-HPLC (purity of all peptides determined to be >90%).

Analytical data are reported in Table 8.

1.1.3. Molecular Dynamics

In the present work, the inventors used a protocol[31] based on the MM/PBSA method[32, 33], where conformations extracted from molecular dynamics simulations are processed using a simplified description for the solvent to yield an estimate of binding free energy. Individual contributions of each amino acid to the complex formation are estimated and important energetic amino acid "hot spots" are identified.

Structures

The initial structure for the apo protein was chain A from the PDB file 1OK7[17], while for the protein and native peptide

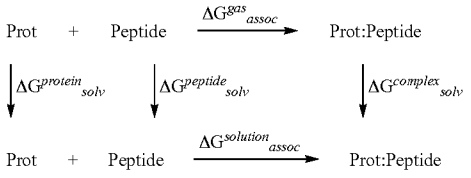
it was chains B and C from the same PDB (1OK7). All crystallographic water molecules were retained.

MD Simulations

The CHARMM program[34], version 32, with the CHARMM 22 all atom protein-nucleic acid force field[35] was used for the molecular dynamics simulations. Hydrogen atoms were added using the HBUILD facility in the CHARMM program. A sphere of 37 Å containing 6840 water molecules (TIP3) was used to solvate the system. Stochastic boundary conditions were imposed and the calculation was limited to residues 7 Å around the peptide. The SHAKE algorithm was used to constrain hydrogen-heavy atom bond distances, and the simulations were done using Langevin algorithm. A 1-fs time step was used for the molecular dynamics simulation and the simulation time. A 12 Å cutoff was used; the van der Waals non bonded terms were treated with a SWITCH potential function whereas the electrostatic terms was evaluated with the SHIFT function.

Free Energy Decomposition of Interactions Between the *E. coli* β Clamp and the Different Peptides.

To obtain a semi-quantitative estimate of the contributions of all amino acids to the binding free energy for the formation of the β clamp-peptide complex, a molecular free energy decomposition scheme based on the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) analysis was performed, following the approach presented by Lafont et al.[31]. From this analysis, an estimation of the free energy of binding for molecular complexes can be obtained. Briefly, in the MM/PBSA approach, the free energy is estimated using a standard thermodynamic cycle of the form



where the binding free energy is calculated according to the equation:

$$\Delta G_{assoc}^{solution} = \Delta E_{MM}^{gas} - T\Delta S_{MM} + \Delta G_{solvation}$$

where Δ_{MM}^{gas} is the difference in the gas phase energy; ΔS_{MM} is the change in entropy upon complex formation and $\Delta G_{solvation}$ is the change in solvation free energy. The gas phase energy differences contain terms for the intermolecular electrostatic and van der Waals energies, as well as the equivalent internal energy terms. These terms are based on the CHARMM force field in the present approach. The solvation free energy is divided into two contributions: an electrostatic and a nonpolar contribution. This latter term is approximated by an empirical relationship based on solvent accessible surface area and the electrostatic contribution is calculated here using the Poisson-Boltzmann equation.

Several approximations are introduced in the MM/PBSA method. The first was the neglect of conformational change upon complex formation, which is dictated by the absence of experimental structures for the unbound protein and peptides. To account for the unbound species in the calculations, their respective structures were obtained from the complex generated during the molecular dynamics simulations. With this approximation, there are no changes to the internal energy terms. The second approximation is the neglect of changes in configuration entropy due to binding. Although these simplifications preclude calculations of absolute values of the binding free energies, they have been shown in previous work to be satisfactory in the context of identifying interaction energy "hot spots" in protein-protein and protein-ligand complexes. Similar simplifications have been employed in other studies[36 31 37]. Via this approach, the total binding free energy can be decomposed into individual energetic contributions per residue. Decomposition of the binding free energy to individual amino acid contributions leads to the identification of amino acids that play a dominant role in binding and can contribute to reliable predictions of the role of particular amino acids in stabilizing complexes.

1.1.4. Structure-Based Design of Peptides

From the initial structural and energetic analysis of the RQLVLGL (SEQ ID No: 1, P1 in Table 1) peptide binding to the β clamp, modification of the sequence appeared potentially interesting in three positions (cf. FIG. **1**): Q2; L3 and the hydrophobic L5-G6-L7 segment. In order to identify interesting modifications, the programs MCSS[38] and SEED[39] were used to dock small librairies of hydrophobic and polar small ligands (fragments) onto the surface of the β-clamp encompassing the peptide binding site. The protocol incorporated improved scoring functions with solvation corrections.[40, 41]. From this initial step, it appeared difficult to find replacements for the Q2 side-chain of the peptide that would correctly maintain the intricate hydrogen-bond network at this position (see FIG. **3**) and therefore no modification of Q2 were attempted. For the other positions, improving interactions with optimized hydrophobic contacts appeared promising. Based on these initial data, a selection of peptides with modified side chains were constructed, docked into the structure and their interactions with the β clamp evaluated using the MM-PBSA protocol described above. The choice of side-chain replacements was based on the docking data, focusing on commercially available protected amino-acids. A force field adapted from CHARMM 22[35] was used for non-natural amino acids. The most promising candidates were selected for synthesis.

1.1.5. β/Peptide Interaction in Solution: In Vitro Competition Assays.

5' end radiolabelling, purification and annealing of synthetic primers were performed as previously described (Wagner et al., 1999). The 30/90mer synthetic construct was obtained by annealing the 30 mer primer (5'GTAAAACGACGGCCAGTGCCAAGCTTAGTC3', SEQ ID No: 34) with the 90 mer template (5 'CCATGATTACGAATTCAGTCATCACCGGCGCCACAGACTAAGCTTGGCACTG GCCGTCGTTTTACAACGTCGTGACTGGGAAAA CCCTGG3', SEQ ID No:35) to form a double stranded structure with 5' and 3' ssDNA overhangs of 25 and 35 nucleotides, respectively. All replication experiments (10 μl final volume) were carried out in buffer E (40 mM HEPES pH 7.5, 80 mM potassium glutamate, 160 μg/ml BSA, 16% glycerol, 0.016% NP40, 8 mM DTT). The 30/90 mer duplex (1 nM final concentration) was first incubated with single strand binding protein (SSB; Sigma; 90 nM final concentration) in the presence of ATP (200 μM) and $MgCl_2$ (7.5 mM) at 37° C. for 10 min. γ complex (1 nM final concentration) (obtained as described by Dallmann et al, 1995) and β clamp (5 nM as dimer final concentration) were added at that stage and incubation was carried out at 37° C. for 10 min. Then, 7 μl of the mixture was added to 1 μl of either DMSO or 1 μl of peptide solution (as specified), incubated 5 min. at room temperature and further 2 hours at 4° C. 1 μl of PolIV was then added (1.5 nM final concentration), incubated 5 min. at room temperature and finally, the whole reaction was mixed with 1 μl of a dNTPs solution (200 μM each dNTP final concentration) and let react for 1 min. at room temperature. Reactions were

quenched by the addition of 20 µl of 95% formamide/dyes solution containing 7.5 mM EDTA, heat-denatured and analysed by chromatography on 12% denaturing polyacrylamide gels. Radiolabelled products were visualised and quantified using a Personal Molecular Imager Fx and the Quantity One software (Bio-Rad).

1.1.6. SPR Assays.

SPR experiments were performed on a Biacore® 3000. The association constant (K_A) of β with the natural Cter heptamer (P1, Table 7) of the DNA polymerase IV of *E. coli* were determined as follow: the β protein (0.125 µM to 2 µM) was injected on the immobilized P1 peptide at a flow rate of 500 $\mu L \cdot min^{-1}$. After subtraction of the background response, the data were fit to the 1:1 Langmuir model using BIAevaluation (Biacore™). The inhibition of P1-β interaction by peptides P2 to P14 (Table 7) was used to measure their affinity for β and was assessed according to the following procedure: complexes of β ring (0.25 µM) with various concentrations of challenging peptides (1.5 nM to 100 µM) were formed and injected on a chip loaded with the P1 peptide. IC_{50} values for each challenging peptide were determined by plotting the concentration of peptide against the percentage of binding inhibition. The IC_{50} value of each peptide was used to calculate Ki ($Ki=(1+K_A[\beta])/IC_{50}$) which measures the affinity of the challenging peptide for β in the competition assay, and AG was derived from Ki ($\Delta G=RT \ln Ki$).

1.1.7. Isothermal Titration Calorimetry.

ITC was performed by using a ITC200 microcalorimeter from MicroCal. Peptides (400 µM) were titrated in sequential injections (2 µl each) into a β ring solution (300 µl, 20 or 30 µM) at 25° C. Data were corrected from control experiments where peptides were injected in buffer solution (Hepes 10 mM pH 7.4, NaCl 0.15M, EDTA 3 mM, P20 0.005%). Data analysis was performed with Origin 7.0 software.

1.1.8. Crystallogenesis, Data Collection and Processing.

Crystallization experiments were essentially conducted as described previously[17]. Crystals of P12-β complexes were grown in capillaries in presence of 0.2% of agarose[42]. Crystallisation buffer contained 100 mM $CaCl_2$, 100 mM Mes pH 6.0 and 30% PEG 400. Cryoprotection was performed by soaking crystals in the same buffer supplemented with 20% glycerol. Cryoprotected crystals were frozen in liquid ethane and X-ray diffraction data were collected at 100 K at beamline X06SA at the Swiss Light Source (Villigen PSI, Switzerland) and beamlines ID29 and ID14-4 at ESRF (Grenoble, France). Diffraction images were processed with XDS, XSCALE and XDSCONV[43]. The structures were solved by molecular replacement with MOLREP[44], using the known beta structure as a search model PDB ID 1OK7[12]. Alternate rounds of rebuilding and refinement, including noncrystallographic symmetry restraints, were carried out with PHENIX[45], COOT[46] and CNS[47]. Model statistics were obtained with Molprobity[48]. Molecular visualizations and structures illustrations were performed using PyMOL[49]. Data processing and refinement statistics are summarized in Table 11.

1.2. Results

1.2.1. Structure and Energetics of the Binding Pocket

The inventors have previously solved the structure of a complex formed between the *E. coli* β clamp and the 16 residues long C-terminal peptide of the *E. coli* DNA polymerase IV (PDB code: 1OK7)[17]. A first part of the present work aimed at unraveling the molecular basis of the peptide-pocket interactions. Molecular modeling approaches were used to determine the contribution of each residue of the last seven amino-acids of the C-terminal part of Pol IV ($R_1Q_2L_3V_4L_5G_6L_7$, SEQ ID No: 32) to the overall interaction (FIG. **2**A), using free energy decomposition (see Material and Methods). For each amino acid, the van der Waals, electrostatic and hydrophobic solvation contribution to binding have been calculated. Stabilizing interactions between the β ring and the peptide are essentially Van der Waals contacts (see FIG. **2**A). Electrostatics contributions are poor, due to compensation between the protein-peptide interaction and the peptide desolvation cost. Hydrophobic solvation contributions are favorable but of lesser magnitude. The net contributions of residues Q2, L3, L5 and L_7 is predominant to the overall interaction (FIG. **2**B). G_6 has no contribution while V_4 which is oriented toward the solvent poorly contributes to the interaction.

Due to the good resolution, the inventors could also analyze the position of water molecules in the free and bound pockets of the 1OK7 structure. In the absence of peptide, four water molecules are located in subsite 1. Upon peptide binding, one is eliminated and one is repositioned close to the T_{142} and Y_{154} residues, allowing the L_5-G_6-L_7 tripeptide to bind into the hydrophobic subsite 1 (FIG. **1**AB). The two water molecules located on the platform in the apo monomer are dislodged upon peptide binding, thus making room for the peptide L_3 residue to bind (FIG. **1**BC). Finally, two water molecules are deeply inserted into the empty subsite 2. One of these two molecules interconnects the $N_\alpha H$ of N_{320} and the $C_\alpha=O$ of M_{362} (FIG. **3**) and is not exchanged with the solvent upon peptide binding, underlining its structural function. The second water molecule is replaced by the Cδ=0 of peptide Q2 residue, while its δ-amino group establishes bounds with the Cα=O of M_{362} and the Cα=O of peptide residue L_2 (FIG. **3**B).

This initial analysis led the inventors to design a minimal peptide binding sequence that was used as a starting point for ligand optimization. Because of the complex network of hydrogen bonds formed by the highly conserved Q residue in subsite 2, one cannot substitute this side chain without dramatically altering the interaction of the whole peptide. Alternatively, several other positions in the peptide sequence may accept modifications that could increase its affinity for the β clamp. Following the structural and energetic analysis of the binding pocket (see Material and Methods), several peptides were synthesized (Table 7 and Table 11) and their binding efficiencies were analyzed by surface plasmon resonance (SPR). The dissociation constant of the P1 natural heptapeptide was measured to be 2.85 (±0.94) 10^{-7} M. As compared to the whole polymerase, this peptide binds 30 fold less efficiently to the ring (table 1, compare P1 and PolIV), pinpointing the contribution of alternate regions of the enzyme to the interaction[16, 14, 19] Removing the G residue of the terminal tripeptide (-LGL) results in a two to three fold decrease in interaction (table 1, compare P1 and P2), while replacing the terminal tripeptide with the consensus LF dipeptide does not affect the affinity (table 1, compare P1 and P3). However, a FL dipeptide totally disrupts peptide binding (table 1, P4). Substituting F for other aromatic residues (W, Y) at the C-terminal position does not contribute to any increased interaction (data not shown). In order to design the shortest peptide, the first (R_1) residue was also removed, which does not seem to contribute significantly to the binding (table 1, P5, FIGS. **1** and **2**)[17], and the V_4 was replaced by a D residue, as observed in the consensus sequence, in order to increase the solubility of the resulting pentapeptide P5 (QLDLF). Although its affinity for the β ring is low, it was increased by 10 fold upon acetylation (table 1, compare P5 and P6), thus providing a good compromise between interaction efficiency and ligand size.

TABLE 1

Influence of the C-terminal tripeptide sequence and effect of N-terminal acetylation on the interaction of peptide with the *E. coli* β clamp, as measured by SPR experiments.

#	se-quence	IC_{50} (μM)	Ki (10^6 M^{-1})	ΔG (Kcal/ mol)	Seq Id No:
PolIV		0.29	4.7	-9.09	
P1	RQLVLGL	8.85	0.15	-7.06	1
P2	RQLVLL	21.53	0.0063	-6.54	2
P3	RQLVLF	8.62	0.15	-7.04	3
P4	RQLVFL	256	Ø	Ø	4
P5	QLDLF	12.44	0.11	-6.87	5
P6	AcQLDLF	1.12	1.2	-8.22	6

Ø: not determined.

Ki = $(1 + K_A[\beta])/IC_{50}$.

ΔG = -RT ln Ki.

PolIV: *E coli* DNA polymerase IV.

1.2.2. Crystal Structure of the P6-β Ring Complex.

The P6 peptide (AcQLDLF, SEQ ID No: 6) co-crystallized with the β ring in conditions similar to those previously described[17] but the cell parameters lead to a V_M value of 7.8, which corresponds to the presence of 3 dimers per asymmetric unit (Table 2). This structure was solved by molecular replacement at 2.3 Å resolution, using our previously determined structure (PDB 1OK7). The superposition of main chain atoms of each ring to the model led to rmsd values ranging from 0.70 Å to 1.06 Å, underlining the close structural similarity of each dimer. Each monomer of the three rings binds a peptide, and all ligands adopt a similar conformation in all six hydrophobic pockets, as indicated by a rmsd value ranging between 0.25 Å to 0.51 Å.

TABLE 2

Statistics on Data Collection and Refinement (related to FIG. 5).

Structure		Beta-P14 (PDB 3Q4L)	Beta-P6 (PDB 3Q4J)	Beta-P12 (PDB 3Q4K)
Data Collection				
Space Group		P1	P1	P1
Unit cell	a (Å)	34.84	35.09	36.25
	b (Å)	79.57	132.87	80
	c (Å)	81.64	137.27	82.18
	α (°)	65.28	62.73	66.15
	β (°)	75.26	88.51	74.94
	γ (°)	82.22	89.77	82.03
Beamline		ID29/ESRF	X06SA/SLS	ID14-4/ESRF
Wavelength (Å)		0.97623	0.915694	0.9794
Resolution limits (Å)		39.2-1.95	29.5-2.3	19.9-2.6
high resolution shell		2.0-1.95	2.35-2.3	2.65-2.6
Reflections:				
measured		221062	296785	42708
unique		54138	96508	22982
Completeness (%)		96.3 (93.8)*	98.6 (97.9)*	91.9 (60.5)*
R_{merge}		0.06 (0.72)*	0.05 (0.36)*	0.067 (0.11)*
I/σ		13 (1.9)*	22.6 (3.6)*	8.7 (4.0)*
Refinement				
Reflections R_{cryst}/R_{free}		54134/2750	96493/7742	22979/1160
R_{cryst} (%)		20.1	21.6	25.9
R_{free} (%)†		23.2	25.0	30.6
Protein atoms		5579	17085	5471
Ligand atoms		106	196	103
Water molecules		299	357	129
Average B factor ($Å^2$)				
Protein		33.3	52.1	30.4
Ligand		39.4	66.5	27.1
Water		40.1	44.5	27.6
R.m.s.d. bond length (Å)		0.01	0.009	0.008
R.m.s.d. angles length (°)		1.13	1.15	1.11

*Values in parentheses correspond to high resolution shell in data collections.

†5% of the reflections were set aside for an Rfree test before initiating any refinement

The atomic coordinates of the peptide and the peptide binding site of the β clamp (residues ≤5 Å from the ligand) are disclosed in the following Table 3. The other residues have the same positions as in the previously determined structure (PDB 1OK7) also described in U.S. Pat. No. 7,635,583.

TABLE 3

Atomic coordinates of P6 residues and of the residues involved in the binding of P6 to the β clamp, in the crystal of P6 peptide co-crystallized with the β ring.

ATOM	1	NE	ARG	B	152	10.195	-25.903	12.978	1.00	60.02	N
ATOM	2	CZ	ARG	B	152	9.832	-25.010	13.891	1.00	70.75	C
ATOM	3	NH1	ARG	B	152	10.045	-23.710	13.686	1.00	51.13	N1+
ATOM	4	NH2	ARG	B	152	9.228	-25.402	15.004	1.00	66.21	N
TER	5		ARG	B	152						
ATOM	6	CG	LEU	B	155	6.034	-25.353	10.551	1.00	37.71	C
ATOM	7	CD1	LEU	B	155	6.887	-24.861	11.676	1.00	37.67	C
ATOM	8	CD2	LEU	B	155	4.805	-25.976	11.115	1.00	31.46	C
TER	9		LEU	B	155						
ATOM	10	CB	THR	B	172	1.710	-23.748	14.242	1.00	31.63	C
ATOM	11	CG2	THR	B	172	2.028	-25.084	13.527	1.00	27.98	C
ATOM	12	OG1	THR	B	172	2.665	-23.450	15.241	1.00	32.30	O
ATOM	13	C	ASP	B	173	6.157	-21.665	14.133	1.00	35.44	C
ATOM	14	N	GLY	B	174	5.672	-22.551	14.996	1.00	34.81	N
ATOM	15	CA	GLY	B	174	6.511	-23.182	16.011	1.00	35.62	C
ATOM	16	C	GLY	B	174	6.492	-22.492	17.359	1.00	39.39	C
ATOM	17	O	GLY	B	174	6.970	-23.064	18.344	1.00	39.92	O
ATOM	18	N	HIS	B	175	5.986	-21.242	17.411	1.00	34.18	N
ATOM	19	CA	HIS	B	175	5.900	-20.479	18.650	1.00	33.72	C
ATOM	20	C	HIS	B	175	4.476	-20.329	19.088	1.00	35.43	C
ATOM	21	O	HIS	B	175	4.175	-20.368	20.282	1.00	34.79	O
ATOM	22	CB	HIS	B	175	6.562	-19.119	18.513	1.00	36.12	C

TABLE 3-continued

Atomic coordinates of P6 residues and of the residues involved in the binding of P6 to the β clamp, in the crystal of P6 peptide co-crystallized with the β ring.

ATOM	23	CG	HIS	B	175	7.984	−19.194	18.096	1.00	41.64	C
ATOM	24	CD2	HIS	B	175	9.032	−19.835	18.668	1.00	44.85	C
ATOM	25	ND1	HIS	B	175	8.394	−18.617	16.936	1.00	45.18	N
ATOM	26	CE1	HIS	B	175	9.678	−18.899	16.829	1.00	45.14	C
ATOM	27	NE2	HIS	B	175	10.115	−19.589	17.878	1.00	45.20	N
ATOM	28	N	ARG	B	176	3.593	−20.133	18.121	1.00	31.08	N
ATOM	29	CA	ARG	B	176	2.181	−19.986	18.371	1.00	29.68	C
ATOM	30	C	ARG	B	176	1.413	−20.789	17.353	1.00	32.45	C
ATOM	31	O	ARG	B	176	1.918	−21.053	16.262	1.00	32.86	O
ATOM	32	N	LEU	B	177	0.240	−21.239	17.733	1.00	27.89	N
ATOM	33	CA	LEU	B	177	−0.619	−22.029	16.875	1.00	27.83	C
ATOM	34	CB	LEU	B	177	−0.579	−23.523	17.307	1.00	27.38	C
ATOM	35	CG	LEU	B	177	−1.466	−24.512	16.510	1.00	31.18	C
ATOM	36	CD1	LEU	B	177	−0.745	−25.845	16.280	1.00	30.87	C
TER	37		LEU	B	177						
ATOM	38	CB	PRO	B	242	3.262	−29.933	14.003	1.00	42.35	C
ATOM	39	CG	PRO	B	242	3.185	−28.774	13.112	1.00	45.82	C
ATOM	40	CD	PRO	B	242	3.325	−29.320	11.745	1.00	40.86	C
TER	41		PRO	B	242						
ATOM	42	O	VAL	B	247	−0.058	−27.602	22.929	1.00	52.04	O
ATOM	43	CB	VAL	B	247	0.470	−28.149	19.728	1.00	50.73	C
ATOM	44	CG1	VAL	B	247	0.574	−26.655	20.015	1.00	50.50	C
ATOM	45	CG2	VAL	B	247	1.641	−28.626	18.875	1.00	51.23	C
TER	46		VAL	B	247						
ATOM	47	O	GLY	B	318	5.474	−15.393	28.086	1.00	28.75	O
ATOM	48	N	PHE	B	319	5.344	−13.225	27.583	1.00	24.23	N
ATOM	49	CA	PHE	B	319	4.851	−13.489	26.241	1.00	24.99	C
ATOM	50	C	PHE	B	319	5.356	−12.468	25.290	1.00	30.49	C
ATOM	51	O	PHE	B	319	5.591	−11.324	25.669	1.00	32.74	O
ATOM	52	CB	PHE	B	319	3.310	−13.478	26.174	1.00	27.25	C
ATOM	53	CG	PHE	B	319	2.640	−14.732	26.670	1.00	28.63	C
ATOM	54	CD1	PHE	B	319	2.741	−15.919	25.957	1.00	30.47	C
ATOM	55	CE1	PHE	B	319	2.128	−17.094	26.424	1.00	31.60	C
ATOM	56	N	ASN	B	320	5.468	−12.865	24.025	1.00	26.27	N
ATOM	57	CA	ASN	B	320	5.720	−11.953	22.949	1.00	26.75	C
ATOM	58	C	ASN	B	320	4.315	−11.306	22.760	1.00	28.81	C
ATOM	59	O	ASN	B	320	3.351	−11.990	22.409	1.00	25.74	O
ATOM	60	CB	ASN	B	320	6.143	−12.740	21.690	1.00	31.09	C
ATOM	61	CG	ASN	B	320	6.252	−11.902	20.458	1.00	38.50	C
ATOM	62	ND2	ASN	B	320	7.226	−12.202	19.631	1.00	36.32	N
TER	63		ASN	B	320						
ATOM	64	CB	TYR	B	323	2.398	−14.188	20.062	1.00	29.74	C
ATOM	65	CG	TYR	B	323	3.671	−14.541	19.312	1.00	34.89	C
ATOM	66	CD2	TYR	B	323	4.613	−15.405	19.867	1.00	36.98	C
ATOM	67	CE2	TYR	B	323	5.769	−15.758	19.176	1.00	38.52	C
ATOM	68	CZ	TYR	B	323	5.978	−15.280	17.899	1.00	45.00	C
ATOM	69	OH	TYR	B	323	7.102	−15.660	17.220	1.00	51.04	O
TER	70		TYR	B	323						
ATOM	71	O	SER	B	343	6.499	−19.652	31.418	1.00	42.19	O
ATOM	72	CA	VAL	B	344	7.142	−22.358	31.029	1.00	30.95	C
ATOM	73	C	VAL	B	344	6.382	−23.225	30.039	1.00	37.16	C
ATOM	74	O	VAL	B	344	6.960	−23.833	29.135	1.00	38.77	O
ATOM	75	CB	VAL	B	344	8.406	−23.037	31.630	1.00	34.02	C
ATOM	76	CG1	VAL	B	344	9.318	−22.002	32.284	1.00	33.14	C
TER	77		VAL	B	344						
ATOM	78	CB	SER	B	346	1.690	−23.500	25.230	1.00	34.25	C
ATOM	79	OG	SER	B	346	0.915	−24.661	25.493	1.00	39.01	O
TER	80		SER	B	346						
ATOM	81	C	VAL	B	360	−0.613	−20.918	21.452	1.00	27.37	C
ATOM	82	O	VAL	B	360	−0.111	−20.800	20.340	1.00	24.84	O
ATOM	83	CB	VAL	B	360	−1.624	−23.300	21.499	1.00	27.65	C
ATOM	84	CG1	VAL	B	360	−0.575	−23.807	22.494	1.00	27.63	C
ATOM	85	C	VAL	B	361	1.982	−19.886	23.474	1.00	27.90	C
ATOM	86	CG1	VAL	B	361	1.873	−16.988	22.556	1.00	22.99	C
ATOM	87	N	MET	B	362	3.180	−20.112	23.023	1.00	28.61	N
ATOM	88	CA	MET	B	362	4.274	−20.561	23.871	1.00	28.56	C
ATOM	89	C	MET	B	362	4.839	−19.321	24.530	1.00	31.58	C
ATOM	90	O	MET	B	362	5.039	−18.292	23.870	1.00	29.49	O
ATOM	91	CB	MET	B	362	5.340	−21.302	23.049	1.00	31.41	C
ATOM	92	CG	MET	B	362	6.222	−22.193	23.888	1.00	35.60	C
ATOM	93	SD	MET	B	362	5.377	−23.603	24.664	1.00	38.96	S
ATOM	94	CE	MET	B	362	6.619	−24.060	25.847	1.00	34.70	C
ATOM	95	N	PRO	B	363	5.071	−19.362	25.842	1.00	29.24	N
ATOM	96	CA	PRO	B	363	5.609	−18.178	26.510	1.00	28.99	C
ATOM	97	C	PRO	B	363	7.074	−17.892	26.226	1.00	33.83	C
ATOM	98	O	PRO	B	363	7.743	−18.614	25.456	1.00	33.34	O
ATOM	99	CB	PRO	B	363	5.341	−18.479	27.991	1.00	30.94	C

TABLE 3-continued

Atomic coordinates of P6 residues and of the residues involved in the binding of P6 to the β clamp, in the crystal of P6 peptide co-crystallized with the β ring.

ATOM	100	CG	PRO	B	363	5.412	−19.947	28.091	1.00	35.37	C
ATOM	101	CD	PRO	B	363	4.870	−20.473	26.798	1.00	31.25	C
ATOM	102	N	MET	B	364	7.545	−16.777	26.784	1.00	29.71	N
ATOM	103	CA	MET	B	364	8.945	−16.382	26.731	1.00	30.02	C
ATOM	104	C	MET	B	364	9.502	−16.624	28.124	1.00	40.88	C
ATOM	105	O	MET	B	364	8.772	−16.499	29.120	1.00	40.62	O
ATOM	106	CB	MET	B	364	9.118	−14.915	26.403	1.00	30.48	C
ATOM	107	CG	MET	B	364	8.757	−14.585	25.034	1.00	32.54	C
ATOM	108	SD	MET	B	364	8.724	−12.808	24.682	1.00	35.07	s
ATOM	109	CE	MET	B	364	10.528	−12.292	24.937	1.00	31.33	C
ATOM	110	N	ARG	B	365	10.767	−17.037	28.190	1.00	42.29	N
ATOM	111	CA	ARG	B	365	11.463	−17.315	29.447	1.00	44.24	C
ATOM	112	C	ARG	B	365	11.620	−15.996	30.209	1.00	47.68	C
ATOM	113	O	ARG	B	365	12.039	−14.991	29.621	1.00	46.53	O
ATOM	114	CB	ARG	B	365	12.812	−18.035	29.173	1.00	49.79	C
ATOM	115	CG	ARG	B	365	13.354	−18.871	30.335	1.00	61.58	C
ATOM	116	CD	ARG	B	365	12.589	−20.152	30.620	1.00	75.54	C
ATOM	117	NE	ARG	B	365	13.073	−21.279	29.817	1.00	90.27	N
ATOM	118	CZ	ARG	B	365	12.957	−22.563	30.161	1.00	100.27	C
ATOM	119	NH1	ARG	B	365	13.409	−23.516	29.355	1.00	79.16	N1+
ATOM	120	NH2	ARG	B	365	12.398	−22.901	31.318	1.00	86.12	N
TER	121		ARG	B	365						
HETATM	122	O	HOH	B	384	8.833	−14.385	20.130	1.00	33.25	O
HETATM	123	O	HOH	B	407	10.652	−12.727	21.066	1.00	31.86	O
HETATM	124	O	HOH	B	465	12.648	−14.060	22.219	1.00	36.65	O
HETATM	125	O	HOH	B	466	13.941	−12.371	23.870	1.00	26.96	O
HETATM	126	C	ACE	H	69	12.190	−16.728	25.287	1.00	47.99	C
HETATM	127	O	ACE	H	69	11.809	−17.683	25.955	1.00	46.88	O
HETATM	128	CH3	ACE	H	69	13.141	−15.743	25.924	1.00	48.05	C
ATOM	129	N	GLN	H	70	11.778	−16.484	24.012	1.00	43.64	N
ATOM	130	CA	GLN	H	70	10.826	−17.283	23.246	1.00	42.45	C
ATOM	131	C	GLN	H	70	11.026	−18.818	23.243	1.00	48.79	C
ATOM	132	O	GLN	H	70	11.987	−19.340	22.644	1.00	49.83	O
ATOM	133	CB	GLN	H	70	10.668	−16.743	21.816	1.00	43.26	C
ATOM	134	CG	GLN	H	70	9.503	−17.399	21.019	1.00	48.43	C
ATOM	135	CD	GLN	H	70	8.133	−17.259	21.688	1.00	51.59	C
ATOM	136	NE2	GLN	H	70	7.769	−16.030	22.096	1.00	32.22	N
ATOM	137	OE1	GLN	H	70	7.418	−18.250	21.901	1.00	38.15	O
ATOM	138	N	LEU	H	71	10.077	−19.531	23.889	1.00	44.09	N
ATOM	139	CA	LEU	H	71	10.074	−20.993	23.930	1.00	42.82	C
ATOM	140	C	LEU	H	71	9.337	−21.541	22.690	1.00	47.21	C
ATOM	141	O	LEU	H	71	8.603	−20.812	22.008	1.00	46.65	O
ATOM	142	CB	LEU	H	71	9.507	−21.552	25.254	1.00	42.99	C
ATOM	143	CG	LEU	H	71	10.264	−21.176	26.550	1.00	48.52	C
ATOM	144	CD1	LEU	H	71	9.369	−21.316	27.773	1.00	48.02	C
ATOM	145	CD2	LEU	H	71	11.512	−22.045	26.736	1.00	53.09	C
ATOM	146	N	ASP	H	72	9.557	−22.813	22.379	1.00	45.35	N
ATOM	147	CA	ASP	H	72	8.966	−23.458	21.213	1.00	45.33	C
ATOM	148	C	ASP	H	72	7.805	−24.369	21.558	1.00	46.72	C
ATOM	149	O	ASP	H	72	7.847	−25.031	22.589	1.00	44.98	O
ATOM	150	CB	ASP	H	72	10.057	−24.179	20.397	1.00	47.71	C
ATOM	151	CG	ASP	H	72	10.805	−23.219	19.472	1.00	73.93	C
ATOM	152	OD1	ASP	H	72	11.558	−22.358	19.986	1.00	75.90	O
ATOM	153	OD2	ASP	H	72	10.576	−23.278	18.231	1.00	87.47	O1−
ATOM	154	N	LEU	H	73	6.766	−24.394	20.694	1.00	44.17	N
ATOM	155	CA	LEU	H	73	5.598	−25.260	20.839	1.00	44.82	C
ATOM	156	C	LEU	H	73	5.949	−26.725	20.585	1.00	49.95	C
ATOM	157	O	LEU	H	73	5.343	−27.628	21.189	1.00	49.17	O
ATOM	158	CB	LEU	H	73	4.487	−24.829	19.868	1.00	44.08	C
ATOM	159	CG	LEU	H	73	3.484	−23.817	20.345	1.00	45.47	C
ATOM	160	CD1	LEU	H	73	2.433	−23.649	19.295	1.00	45.34	C
ATOM	161	CD2	LEU	H	73	2.807	−24.251	21.651	1.00	38.54	C
ATOM	162	N	PHE	H	74	6.897	−26.941	19.644	1.00	47.70	N
ATOM	163	CA	PHE	H	74	7.378	−28.265	19.211	1.00	48.65	C
ATOM	164	C	PHE	H	74	8.752	−28.089	18.510	1.00	75.49	C
ATOM	165	O	PHE	H	74	9.100	−26.937	18.126	1.00	77.08	O
ATOM	166	CB	PHE	H	74	6.340	−28.932	18.271	1.00	49.99	C
ATOM	167	CG	PHE	H	74	5.819	−28.030	17.171	1.00	50.87	C
ATOM	168	CD1	PHE	H	74	6.502	−27.909	15.963	1.00	52.73	C
ATOM	169	CD2	PHE	H	74	4.661	−27.281	17.352	1.00	52.30	C
ATOM	170	CE1	PHE	H	74	6.047	−27.044	14.972	1.00	52.86	C
ATOM	171	CE2	PHE	H	74	4.230	−26.379	16.375	1.00	53.68	C
ATOM	172	CZ	PHE	H	74	4.918	−26.281	15.186	1.00	51.51	C
ATOM	173	OXT	PHE	H	74	9.469	−29.102	18.345	1.00	100.17	O1−
TER	174		PHE	H	74						

TABLE 3-continued

Atomic coordinates of P6 residues and of the residues involved in the binding of P6 to the β clamp, in the crystal of P6 peptide co-crystallized with the β ring.

HETATM	175	O	HOH	H	86	5.592	−15.725	23.553	1.00	33.16	O
END											

A free energy decomposition analysis (see Material and Methods for details) of this complex was performed (FIG. **2**B) and the most important interactions are similar to the initial complex 1OK7, as expected. The canonical sequence LF advantageously replaces the LGL sequence in C-ter of the peptide (FIG. **2**B). The P6 peptide acetyl group also forms two hydrogen bonds with the Nα of residues R_{365} and L_{366} of the β monomer which probably account for the 10 fold increase in stability of the P6 peptide as compared to P5 (Table 1). Despite its reduced size, the P6 peptide therefore has an increased affinity for the β-clamp with respect to the original peptide P1.

1.2.3. Design of Non-Natural Peptides Ligands with Increased Binding Affinity.

P6 was further used as a lead to introduce modifications aimed at increasing the affinity of the ligand for the β clamp. Because the natural ligand binds to the pocket essentially through hydrophobic interactions, the aim was to extend the network of such interactions. A first set of modifications concerned position 2, where the leucine residue was replaced by a cyclohexyl-L-alanyl group (Cha) (P7, table 4 and table 7). An initial modeling analysis, using programs MCSS and SEED, indicated that this modification provides a Van der Waals energy contribution two-fold higher than that with the natural L_3 residue, and is the most efficient group tested (Table 4). It also results in a 6 fold increase in the interaction, as measured by SPR (Table 4 and Table 7, compare P6 and P7). Attempts to increase the side chain length resulted in a drastic reduction of the affinity (Table 4 and Table 7, P8) while other modifications like homoleucyl (hLeu) or neopentylglycyl (NptGly, also called tertiomethylbutylglycine, tBMG) did not yield any gain in affinity (Table 4 and Table 7, P9 and P10), probably indicating that the area available for an efficient interaction is limited.

TABLE 4

Effect of site-specific modifications of the second residue on the interaction of peptide with the *E. coli* β clamp.

#	sequence	IC_{50} (μM)	SEQ ID No:
P6	AcQLDLF	1.12	6
P7	Ac Q ChaDLF	0.17	7
P8	Ac Q hCha DLF	82.8	8
P9	Ac Q hLeu DLF	0.74	9
P10	Ac Q NptGly DLF	0.99	10

Cha: cyclohexylalanine,
hCha: homocyclohexylalanine,
hLeu: homoleucine,
NptGly: neopentylglycyl.

A second set of similar modifications was introduced at position L_4, but no increase in affinity was observed as compared to P7 (Table 5).

TABLE 5

Effect of site-specific modifications of the L_4, residue on the interaction of peptide with the *E. coli* β clamp.

#	sequence	IC_{50} (μM)	SEQ ID No:
P6	AcQLDLF	1.12	6
P7	Ac Q Cha DLF	0.17	7
P16	Ac Q Cha D hLeu F	0.23	16
P17	Ac Q Cha D Cha F	0.31	17
P18	Ac Q Cha D hCha F	0.72	18
P19	Ac Q ChaD NptGly F	5.63	19

Finally, several modifications were introduced on the terminal phenylalanine benzyl ring (Table 6). The binding affinity was found to increase significantly with the size of the ring substituent (p-methyl<p-chloro<p-bromo<3,4-dichloro)(see P11, P12, P13, and P14, respectively). An IC_{50} value of 70 nM was measured for the 3,4-dichlorophenylalanine containing peptide (P14), which represent a 15, 115 and 4 fold increase as compared to P6, P1 and the full PolIV enzyme, respectively (Table 6 and Table 7). To the contrary, replacement of the terminal phenylalanine by a cyclohexyl-L-alanyl group (Cha), a tryptophan or a 2-amino-tetradecanoic acid (Atda) (P20, P21 and P22, Table 6) led to a decreased affinity.

TABLE 6

Effect of modifications on the terminal phenylalanine benzyl ring on the interaction of peptide with the *E. coli* β clamp.

#	sequence	IC_{50} (μM)	SEQ ID No:
P6	AcQLDLF	1.12	6
P11	Ac-Q Cha DL pMeF	0.26	11
P12	Ac-Q Cha DL pClF	0.16	12
P13	Ac-Q Cha DL pBrF	0.10	13
P14	Ac-Q Cha DL diClF	0.072	14
P20	Ac-Q Cha DL Cha	0.41	20
P21	Ac-Q Cha DLW	0.64	21
P22	Ac-Q Cha DL Atda	3.72	22

The interactions of the various peptides with the β ring were also evaluated using a polymerase competition assay where primer elongation performed by the polIV DNA polymerase is challenged by various concentrations of the peptides to be tested[17]. While the β independent activity of the polymerase is insensitive to the peptides, indicating that they have no direct effect on the enzyme activity, the β dependent elongation activity is differentially inhibited depending on

the affinity of the peptide for its target (FIG. **4**). A quantitative analysis of the biochemical assays is shown in FIG. **7**. Although this approach is less sensitive than SPR, a good correlation is observed between the two methods (FIG. **4**).

TABLE 7

Influence of the C-terminal tripeptide sequence and effect of site specific modifications on the interaction of peptide with the *E. coli* β clamp, as measured by SPR experiments.

#	se-quence	IC_{50} (μM)	Ki (10^6 M^{-1})	ΔG (Kcal/ mol)	Seq Id No:
PolIV		0.29	4.7	-9.09	
P1	RQLVLGL	8.85	0.15	-7.06	1
P2	RQLVLL	21.53	0.0063	-6.54	2
P3	RQLVLF	8.62	0.15	-7.04	3
P4	RQLVFL	256	Ø	Ø	4
P5	QLDLF	12.44	0.11	-6.87	5
P6	AcQLDLF	1.12	1.2	-8.22	6
P7	Ac Q ChaDLF	0.17	8.01	-9.42	7
P8	Ac Q hCha DLF	82.8	16.4	-5.74	8
P9	Ac Q Hol DLF	0.74	1.84	-8.54	9
P10	Ac Q NptGly DLF	0.99	1.36	-8.36	10
P11	Ac-Q Cha DL pMeF	0.26	8.43	-9.44	11
P12	Ac-Q Cha DL pClF	0.16	13.7	-9.73	12
P13	Ac-Q Cha DL pBrF	0.096	13.49	-9.71	13
P14	Ac-Q Cha DL diClF	0.077	17	-9.85	14

Ø: not determined.
Ki = (1 + K_A[β])/IC50.
ΔG = -RT ln Ki.
PolIV: *E coli* DNA polymerase IV.

The following table 8 presents the analytical data concerning the most relevant peptides described in this study.

TABLE 8

Sequence and analytical data of C-terminal peptides of the *E. coli* DNA polymerase IV and analogues (related to FIG. 1 and table 7).

Pep-tide	compound	HPLC t_R (min)	PU-RITY [%] HPLC	MS CALC. MASS	MS FOUND [M + H^+]	SEQ ID No:
P1	H-Arg-Gln-Leu-Val-Leu-Gly-Leu-OH–	11.97	98.2	799.0	799.9	1
P2	H-Arg-Gln-Leu-Val-Leu-Leu-OH	11.50	93.0	740.5	741.9	2
P3	H-Arg-Gln-Leu-Val-Leu-Phe-OH	12.13	92.1	774.5	775.8	3
P4	H-Arg-Gln-Leu-Val-Phe-Leu-OH	12.02	92.5	774.5	775.5	4
P5	H-Gln-Leu-Asp-Leu-Phe-OH	12.55	90	634.3	634.8	5

TABLE 8-continued

Sequence and analytical data of C-terminal peptides of the *E. coli* DNA polymerase IV and analogues (related to FIG. 1 and table 7).

Pep-tide	compound	HPLC t_R (min)	PU-RITY [%] HPLC	MS CALC. MASS	MS FOUND [M + H^+]	SEQ ID No:
P6	Ac-Gln-Leu-Asp-Leu-Phe-OH	13.25	92.5	676.3	677.3	6
P7	Ac-Gln-Cha-Asp-Leu-Phe-OH	15.12	95	716.8	717.7	7
P8	Ac-Gln-hCha-Asp-Leu-Phe-OH	16.23	50.93	730.8	731.4	8
P9	Ac-Gln-Hol-Asp-Leu-Phe-OH	14.23	90	690.8	691.0	9
P10	Ac-Gln-NptGly-Asp-Leu-Phe-OH	13.99	94	702.8	691.7	10
P11	Ac-Gln-Cha-Asp-Leu-*p*MePhe-OH	15.88	97	716.4	732.7	11
P12	Ac-Gln-Cha-Asp-Leu-*p*ClPhe-OH	16.27	93	736.3	752.7	12
P13	Ac-Gln-Cha-Asp-Leu-*p*BrPhe-OH	16.45	95	794.3	799.8	13
P14	Ac-Gln-Cha-Asp-Leu-(3,4-di-Cl)Phe-OH	17.18	91	784.3	786.9	14

Cha: beta-cyclohexyl-L-alanyl;
hCha: L-homoCha;
Hol: L-homoleucyl;
NptGly: neopentylglycyl;
pMePhe: 4-methyl-L-phenylalanyl;
pClPhe: 4-chloro-L-phenylalanyl;
pBrPhe: 4-bromo-L-phenylalanyl;
(3,4-di-Cl)Phe: 3,4-dichloro-L-phenylalanyl

1.2.4. Crystal Structure of the Modified Peptides β Ring Complexes.

Peptides P12 and P14 were co-crystallized with the β ring. The structures were solved by molecular replacement at 2.6 Å and 1.95 Å resolution respectively, using the 1OK7 structure as a search model. As for the previous P6-β complex, both complexes crystallized in space group P1 but with one ring per asymmetric unit (Table 3). Each monomer of the rings binds a peptide, and both ligands adopt a similar configuration (rmsd=0.70 Å and 0.78 Å for the peptides atoms of P12 and P14, respectively), indicating that the modified peptides essentially adopt the same conformation and location in the binding pocket. The Cha moiety is located at the same position as the L_3 residue of P6 but extends its interaction further within a hydrophobic pocket shaped by the P_{363}, V_{344}, M_{362} and R_{365} β residues (FIG. **5**A) and interacts with the last three residues. These extra interactions probably account for the increased affinity of P7 as compared to P6 (table 1). Additionally, the Nα of this modified residue interacts with the Cα=O of P_{363}. The p-chloro and 3,4-dichloro F residues of P12 and P14 are almost superimposed (FIG. **5**B) and interact with β residues T_{172}, L_{177} and V_{247}. The chlorine atom in meta position in P14 establishes an halogen bond with the hydroxyl oxygen of T_{172}, with contact distance and angle in good agreement with previously published data (d=3.17 Å and θ=148.71°)[20]. In contrast, the para chlorine atoms of both P12 and P14 do not establish such type of interaction because

the distances with adjacent oxygen atoms of β residues are too large.

The atomic coordinates P12 and P14, co-crystalized with the β clamp, are disclosed in the following Tables 9 and 10.

These tables indicate the atomic coordinates of the peptides and of the binding site of the β clamp (residues≤5 Å from the ligand). The other residues have the same positions as in the previously determined structure (PDB 1OK7) also described in U.S. Pat. No. 7,635,583.

TABLE 9

Atomic coordinates of P12 residues and of the residues involved in the binding of P12 to the β clamp, in the crystal of P12 peptide co-crystallized with the β ring.

ATOM	1	NH1	ARG	A	152	−13.878	13.827	24.977	1.00	41.13	N1+
TER	2		ARG	A	152						
ATOM	3	CD2	LEU	A	155	−9.376	16.306	22.697	1.00	33.78	C
TER	4		LEU	A	155						
ATOM	5	CB	THR	A	172	−5.579	13.660	24.332	1.00	10.80	C
ATOM	6	CG2	THR	A	172	−6.162	15.062	24.379	1.00	3.00	C
ATOM	7	OG1	THR	A	172	−6.396	12.784	25.111	1.00	11.87	O
TER	8		THR	A	172						
ATOM	9	N	GLY	A	174	−9.375	11.662	24.270	1.00	22.35	N
ATOM	10	CA	GLY	A	174	−10.197	11.573	25.471	1.00	21.94	C
ATOM	11	C	GLY	A	174	−9.904	10.341	26.321	1.00	26.11	C
ATOM	12	O	GLY	A	174	−10.187	10.331	27.522	1.00	24.70	O
ATOM	13	N	HIS	A	175	−9.341	9.286	25.699	1.00	23.67	N
ATOM	14	CA	HIS	A	175	−9.001	8.008	26.347	1.00	23.91	C
ATOM	15	C	HIS	A	175	−7.479	7.869	26.584	1.00	24.57	C
ATOM	16	O	HIS	A	175	−7.046	7.395	27.635	1.00	24.56	O
ATOM	17	CB	HIS	A	175	−9.546	6.822	25.519	1.00	25.86	C
ATOM	18	CG	HIS	A	175	−10.971	7.005	25.080	1.00	30.11	C
ATOM	19	CD2	HIS	A	175	−11.486	7.140	23.838	1.00	32.30	C
ATOM	20	ND1	HIS	A	175	−12.005	7.082	25.997	1.00	32.23	N
ATOM	21	CE1	HIS	A	175	−13.107	7.245	25.288	1.00	31.59	C
ATOM	22	NE2	HIS	A	175	−12.845	7.286	23.985	1.00	32.09	N
ATOM	23	N	ARG	A	176	−6.685	8.290	25.610	1.00	18.69	N
ATOM	24	CA	ARG	A	176	−5.234	8.264	25.702	1.00	18.59	C
ATOM	25	C	ARG	A	176	−4.604	9.609	25.304	1.00	24.08	C
ATOM	26	O	ARG	A	176	−5.276	10.463	24.713	1.00	23.83	O
ATOM	27	N	LEU	A	177	−3.347	9.832	25.715	1.00	19.54	N
ATOM	28	CA	LEU	A	177	−2.653	11.088	25.503	1.00	18.04	C
ATOM	29	CB	LEU	A	177	−2.873	11.987	26.745	1.00	17.92	C
ATOM	30	CG	LEU	A	177	−1.963	13.211	26.971	1.00	21.84	C
ATOM	31	CD1	LEU	A	177	−2.328	14.347	26.066	1.00	21.30	C
ATOM	32	CD2	LEU	A	177	−2.004	13.663	28.416	1.00	22.36	C
TER	33		LEU	A	177						
ATOM	34	CA	PRO	A	242	−8.284	20.266	27.211	1.00	10.30	C
ATOM	35	C	PRO	A	242	−7.012	20.416	28.076	1.00	16.74	C
ATOM	36	CB	PRO	A	242	−8.798	18.838	27.188	1.00	11.44	C
ATOM	37	CG	PRO	A	242	−8.164	18.255	25.944	1.00	16.16	C
ATOM	38	CD	PRO	A	242	−8.332	19.361	24.955	1.00	11.62	C
ATOM	39	N	ASP	A	243	−7.224	20.463	29.391	1.00	14.24	N
ATOM	40	CA	ASP	A	243	−6.222	20.608	30.438	1.00	14.52	C
ATOM	41	C	ASP	A	243	−5.454	19.282	30.618	1.00	21.86	C
ATOM	42	O	ASP	A	243	−5.842	18.424	31.416	1.00	25.37	O
ATOM	43	N	TYR	A	244	−4.471	19.055	29.748	1.00	15.95	N
ATOM	44	CA	TYR	A	244	−3.688	17.827	29.764	1.00	15.04	C
ATOM	45	CB	TYR	A	244	−2.900	17.645	28.437	1.00	14.90	C
TER	46		TYR	A	244						
ATOM	47	NH1	ARG	A	246	−9.401	15.913	36.324	1.00	21.44	N1+
ATOM	48	CB	VAL	A	247	−4.905	13.902	32.710	1.00	29.59	C
ATOM	49	CG1	VAL	A	247	−5.480	12.551	33.104	1.00	29.67	C
ATOM	50	CG2	VAL	A	247	−6.024	14.870	32.343	1.00	29.06	C
TER	51		VAL	A	247						
ATOM	52	O	PHE	A	278	−11.396	−2.943	23.858	1.00	27.53	O
TER	53		PHE	A	278						
ATOM	54	N	ASN	A	320	−7.095	−1.216	26.969	1.00	26.00	N
ATOM	55	CB	ASN	A	320	−8.050	−0.275	24.887	1.00	19.44	C
ATOM	56	CG	ASN	A	320	−8.114	−0.289	23.378	1.00	25.87	C
ATOM	57	ND2	ASN	A	320	−9.197	0.247	22.826	1.00	19.30	N
ATOM	58	OD1	ASN	A	320	−7.179	−0.699	22.685	1.00	16.42	O
TER	59		ASN	A	320						
ATOM	60	CD2	TYR	A	323	−6.890	3.229	24.491	1.00	20.56	C
ATOM	61	CE2	TYR	A	323	−8.089	3.767	24.028	1.00	21.88	C
ATOM	62	cz	TYR	A	323	−8.254	4.015	22.675	1.00	30.51	C
ATOM	63	OH	TYR	A	323	−9.422	4.548	22.199	1.00	34.44	O
TER	64		TYR	A	323						
ATOM	65	O	SER	A	343	−8.420	0.538	36.986	1.00	43.35	O
ATOM	66	CA	VAL	A	344	−9.412	2.967	38.022	1.00	37.68	C
ATOM	67	C	VAL	A	344	−8.749	4.286	37.541	1.00	39.37	C
ATOM	68	O	VAL	A	344	−9.430	5.162	37.004	1.00	37.60	O

TABLE 9-continued

Atomic coordinates of P12 residues and of the residues involved in the binding of P12 to the β clamp, in the crystal of P12 peptide co-crystallized with the β ring.

ATOM	69	CB	VAL	A	344	−10.716	3.216	38.843	1.00	41.47	C
ATOM	70	CG1	VAL	A	344	−11.660	2.025	38.749	1.00	41.11	C
TER	71		VAL	A	344						
ATOM	72	CB	SER	A	346	−4.248	7.581	33.672	1.00	36.35	C
TER	73		SER	A	346						
ATOM	74	O	VAL	A	360	−2.861	8.288	27.992	1.00	21.29	O
ATOM	75	CG1	VAL	A	360	−2.051	9.515	31.551	1.00	22.57	C
TER	76		VAL	A	360						
ATOM	77	N	MET	A	362	−5.771	5.847	29.897	1.00	24.01	N
ATOM	78	CA	MET	A	362	−6.824	5.610	30.866	1.00	25.90	C
ATOM	79	C	MET	A	362	−7.277	4.123	30.792	1.00	30.54	C
ATOM	80	O	MET	A	362	−7.461	3.593	29.689	1.00	29.30	O
ATOM	81	CB	MET	A	362	−7.994	6.560	30.587	1.00	29.26	C
ATOM	82	CG	MET	A	362	−8.871	6.790	31.773	1.00	34.65	C
ATOM	83	SD	MET	A	362	−8.107	7.741	33.104	1.00	40.06	s
ATOM	84	CE	MET	A	362	−9.291	7.371	34.427	1.00	36.64	C
ATOM	85	N	PRO	A	363	−7.464	3.423	31.935	1.00	28.36	N
ATOM	86	CA	PRO	A	363	−7.885	2.016	31.872	1.00	27.57	C
ATOM	87	C	PRO	A	363	−9.367	1.784	31.553	1.00	32.79	C
ATOM	88	O	PRO	A	363	−10.161	2.723	31.445	1.00	30.76	O
ATOM	89	CB	PRO	A	363	−7.534	1.508	33.276	1.00	29.22	C
ATOM	90	CG	PRO	A	363	−7.733	2.646	34.141	1.00	33.99	C
ATOM	91	CD	PRO	A	363	−7.288	3.852	33.338	1.00	30.19	C
ATOM	92	N	MET	A	364	−9.735	0.505	31.409	1.00	33.12	N
ATOM	93	CA	MET	A	364	−11.116	0.063	31.233	1.00	34.28	C
ATOM	94	C	MET	A	364	−11.510	−0.892	32.363	1.00	38.91	C
ATOM	95	CB	MET	A	364	−11.392	−0.494	29.827	1.00	37.35	C
ATOM	96	CG	MET	A	364	−10.591	−1.704	29.437	1.00	42.38	C
ATOM	97	SD	MET	A	364	−10.644	−1.982	27.634	1.00	48.21	s
ATOM	98	CE	MET	A	364	−12.355	−2.535	27.415	1.00	45.40	C
ATOM	99	N	ARG	A	365	−12.802	−0.893	32.770	1.00	36.16	N
ATOM	100	CA	ARG	A	365	−13.345	−1.727	33.857	1.00	54.18	C
ATOM	101	O	ARG	A	365	−13.715	−3.627	32.448	1.00	15.77	O
ATOM	102	CB	ARG	A	365	−14.777	−1.280	34.190	1.00	54.40	C
ATOM	103	CG	ARG	A	365	−14.987	−0.808	35.628	1.00	61.81	C
ATOM	104	CD	ARG	A	365	−16.323	−0.093	35.795	1.00	67.50	C
ATOM	105	NE	ARG	A	365	−16.385	0.668	37.050	1.00	70.43	N
ATOM	106	CZ	ARG	A	365	−17.177	1.718	37.255	1.00	69.73	C
ATOM	107	NH1	ARG	A	365	−17.980	2.151	36.291	1.00	47.59	N1+
ATOM	108	NH2	ARG	A	365	−17.162	2.350	38.424	1.00	45.96	N
TER	109		ARG	A	365						
HETATM	110	O	HOH	A	397	−12.948	−0.572	24.569	1.00	24.99	O
HETATM	111	O	HOH	A	422	−15.206	5.096	38.996	1.00	47.81	O
HETATM	112	O	HOH	A	433	−8.020	1.912	27.730	1.00	9.25	O
HETATM	113	O	HOH	C	24	−11.224	1.716	24.238	1.00	30.48	O
HETATM	114	C	ACE	C	367	−14.628	0.704	29.591	1.00	35.56	C
HETATM	115	O	ACE	C	367	−14.221	1.136	30.667	1.00	30.52	O
HETATM	116	CH3	ACE	C	367	−15.612	−0.454	29.524	1.00	29.87	C
ATOM	117	N	GLN	C	368	−14.292	1.401	28.359	1.00	30.56	N
ATOM	118	CA	GLN	C	368	−13.424	2.699	28.412	1.00	25.45	C
ATOM	119	C	GLN	C	368	−13.778	3.790	29.559	1.00	32.24	C
ATOM	120	O	GLN	C	368	−14.830	4.442	29.488	1.00	32.94	O
ATOM	121	CB	GLN	C	368	−13.142	3.398	26.993	1.00	29.55	C
ATOM	122	CG	GLN	C	368	−11.862	4.252	26.986	1.00	29.78	C
ATOM	123	CD	GLN	C	368	−10.609	3.491	27.350	1.00	23.65	C
ATOM	124	NE2	GLN	C	368	−9.792	3.893	28.463	1.00	24.50	N
ATOM	125	OE1	GLN	C	368	−10.426	2.411	26.809	1.00	27.24	O
TER	126		GLN	C	368						
HETATM	127	N	ALC	C	369	−12.687	4.147	30.488	1.00	28.64	N
HETATM	128	CA	ALC	C	369	−12.514	5.508	30.964	1.00	29.77	C
HETATM	129	C	ALC	C	369	−11.636	6.300	29.995	1.00	30.06	C
HETATM	130	O	ALC	C	369	−10.844	5.719	29.263	1.00	32.57	O
HETATM	131	CB	ALC	C	369	−11.826	5.516	32.374	1.00	29.41	C
HETATM	132	CG	ALC	C	369	−12.462	4.800	33.632	1.00	27.38	C
HETATM	133	CD1	ALC	C	369	−11.611	3.659	34.076	1.00	26.63	C
HETATM	134	CD2	ALC	C	369	−13.892	4.431	33.398	1.00	29.06	C
HETATM	135	CE1	ALC	C	369	−12.176	2.854	35.208	1.00	26.40	C
HETATM	136	CE2	ALC	C	369	−14.487	3.799	34.618	1.00	36.93	C
HETATM	137	cz	ALC	C	369	−13.665	2.730	35.276	1.00	30.08	C
ATOM	138	N	ASP	C	370	−11.984	7.629	29.488	1.00	34.94	N
ATOM	139	CA	ASP	C	370	−12.550	8.830	30.301	1.00	34.00	C
ATOM	140	C	ASP	C	370	−11.474	9.729	31.120	1.00	30.52	C
ATOM	141	O	ASP	C	370	−11.377	9.697	32.347	1.00	25.64	O
ATOM	142	CB	ASP	C	370	−14.029	8.546	30.909	1.00	38.94	C
ATOM	143	CG	ASP	C	370	−15.012	8.727	29.752	1.00	40.34	C
ATOM	144	OD1	ASP	C	370	−16.182	8.114	29.765	1.00	59.35	O
ATOM	145	OD2	ASP	C	370	−14.418	9.287	28.668	1.00	30.05	O1−

TABLE 9-continued

Atomic coordinates of P12 residues and of the residues involved in the binding of P12 to the β clamp, in the crystal of P12 peptide co-crystallized with the β ring.

ATOM	146	N	LEU	C	371	−10.560	10.539	30.269	1.00	32.14	N
ATOM	147	CA	LEU	C	371	−9.624	11.571	30.769	1.00	33.35	C
ATOM	148	C	LEU	C	371	−10.269	12.914	31.093	1.00	36.56	C
ATOM	149	O	LEU	C	371	−9.703	13.671	31.882	1.00	38.88	O
ATOM	150	CB	LEU	C	371	−8.465	11.790	29.754	1.00	29.71	C
ATOM	151	CG	LEU	C	371	−7.489	10.663	29.542	1.00	32.92	C
ATOM	152	CD1	LEU	C	371	−6.748	10.753	28.242	1.00	21.26	C
ATOM	153	CD2	LEU	C	371	−6.637	10.343	30.752	1.00	23.07	C
TER	154		LEU	C	371						
HETATM	155	N	200	C	372	−11.514	13.352	30.399	1.00	31.07	N
HETATM	156	CA	200	C	372	−12.085	14.735	30.622	1.00	32.30	C
HETATM	157	C	200	C	372	−13.594	14.731	30.756	1.00	37.62	C
HETATM	158	O	200	C	372	−14.200	15.440	31.810	1.00	41.91	O
HETATM	159	CB	200	C	372	−11.640	15.817	29.612	1.00	30.34	C
HETATM	160	CG	200	C	372	−10.185	15.711	29.135	1.00	25.13	C
HETATM	161	CD1	200	C	372	−9.909	15.210	27.863	1.00	20.80	C
HETATM	162	CD2	200	C	372	−9.161	16.082	29.995	1.00	22.71	C
HETATM	163	CE1	200	C	372	−8.592	15.088	27.455	1.00	22.75	C
HETATM	164	CE2	200	C	372	−7.842	15.960	29.593	1.00	22.47	C
HETATM	165	CZ	200	C	372	−7.572	15.459	28.324	1.00	26.57	C
HETATM	166	CL	200	C	372	−5.931	15.288	27.829	1.00	37.22	CL
HETATM	167	OXT	200	C	372	−14.362	13.982	29.828	1.00	44.19	O
END											

TABLE 10

Atomic coordinates of P14 residues and of the residues involved in the binding of P14 to the β clamp, in the crystal of P14 peptide co-crystallized with the β ring.

ATOM	1	NH1	ARG	A	152	−13.000	14.676	25.384	1.00	41.87	N1+
TER	2		ARG	A	152						
ATOM	3	CD2	LEU	A	155	−8.771	17.218	22.511	1.00	32.76	C
TER	4		LEU	A	155						
ATOM	5	CB	THR	A	172	−5.062	14.269	24.018	1.00	29.73	C
ATOM	6	CG2	THR	A	172	−5.795	15.620	24.122	1.00	26.88	C
ATOM	7	OG1	THR	A	172	−5.728	13.271	24.786	1.00	28.48	O
TER	8		THR	A	172						
ATOM	9	N	GLY	A	174	−8.679	12.290	24.030	1.00	26.79	N
ATOM	10	CA	GLY	A	174	−9.451	12.319	25.270	1.00	28.12	C
ATOM	11	C	GLY	A	174	−9.367	11.041	26.069	1.00	32.47	C
ATOM	12	O	GLY	A	174	−9.848	10.979	27.199	1.00	31.98	O
ATOM	13	N	HIS	A	175	−8.715	10.024	25.494	1.00	30.75	N
ATOM	14	CA	HIS	A	175	−8.481	8.734	26.139	1.00	30.80	C
ATOM	15	C	HIS	A	175	−6.976	8.530	26.320	1.00	30.70	C
ATOM	16	O	HIS	A	175	−6.557	8.002	27.335	1.00	29.87	O
ATOM	17	CB	HIS	A	175	−9.131	7.602	25.325	1.00	32.00	C
ATOM	18	CG	HIS	A	175	−10.595	7.832	25.079	1.00	35.24	C
ATOM	19	CD2	HIS	A	175	−11.259	7.998	23.916	1.00	37.80	C
ATOM	20	ND1	HIS	A	175	−11.496	7.948	26.130	1.00	37.36	N
ATOM	21	CE1	HIS	A	175	−12.677	8.158	25.576	1.00	37.24	C
ATOM	22	NE2	HIS	A	175	−12.587	8.212	24.247	1.00	38.20	N
ATOM	23	N	ARG	A	176	−6.162	8.980	25.337	1.00	25.93	N
ATOM	24	CA	ARG	A	176	−4.709	8.899	25.425	1.00	23.72	C
ATOM	25	C	ARG	A	176	−4.078	10.213	25.009	1.00	27.35	C
ATOM	26	O	ARG	A	176	−4.648	10.950	24.212	1.00	24.69	O
ATOM	27	N	LEU	A	177	−2.885	10.489	25.534	1.00	25.59	N
ATOM	28	CA	LEU	A	177	−2.204	11.738	25.266	1.00	25.33	C
ATOM	29	CB	LEU	A	177	−2.431	12.671	26.488	1.00	25.95	C
ATOM	30	CG	LEU	A	177	−1.853	14.080	26.442	1.00	29.85	C
ATOM	31	CD1	LEU	A	177	−2.845	15.053	27.040	1.00	31.56	C
TER	32		LEU	A	177						
ATOM	33	CA	PRO	A	242	−7.701	20.996	27.178	1.00	30.57	C
ATOM	34	C	PRO	A	242	−6.353	21.180	27.870	1.00	35.62	C
ATOM	35	O	PRO	A	242	−5.277	21.201	27.239	1.00	31.98	O
ATOM	36	CB	PRO	A	242	−8.234	19.577	27.285	1.00	32.60	C
ATOM	37	CG	PRO	A	242	−7.827	18.915	26.040	1.00	37.83	C
ATOM	38	N	ASP	A	243	−6.480	21.417	29.176	1.00	34.91	N
ATOM	39	CA	ASP	A	243	−5.451	21.642	30.163	1.00	35.21	C
ATOM	40	C	ASP	A	243	−4.812	20.307	30.500	1.00	38.35	C
ATOM	41	O	ASP	A	243	−5.193	19.613	31.455	1.00	37.41	O
ATOM	42	N	TYR	A	244	−3.865	19.930	29.652	1.00	34.76	N
ATOM	43	CA	TYR	A	244	−3.152	18.662	29.719	1.00	34.71	C
ATOM	44	C	TYR	A	244	−2.245	18.565	30.939	1.00	39.89	C
ATOM	45	CB	TYR	A	244	−2.366	18.444	28.424	1.00	34.64	C

TABLE 10-continued

Atomic coordinates of P14 residues and of the residues involved in the binding of P14 to the β clamp, in the crystal of P14 peptide co-crystallized with the β ring.

TER	46		TYR	A	244					
ATOM	47	NH1	ARG	A	246	−9.362	18.335	34.959	1.00	56.46 N1+
ATOM	48	CB	VAL	A	247	−4.800	14.658	32.377	1.00	45.61 C
ATOM	49	CG1	VAL	A	247	−6.175	15.272	32.614	1.00	44.77 C
ATOM	50	CG2	VAL	A	247	−4.260	15.077	31.013	1.00	45.81 C
TER	51		VAL	A	247					
ATOM	52	O	GLY	A	318	−7.101	−0.481	31.341	1.00	28.54 O
ATOM	53	N	PHE	A	319	−6.439	−2.005	29.819	1.00	25.76 N
ATOM	54	CA	PHE	A	319	−6.062	−1.039	28.794	1.00	25.29 C
ATOM	55	C	PHE	A	319	−6.489	−1.419	27.399	1.00	26.95 C
ATOM	56	O	PHE	A	319	−6.510	−2.591	27.065	1.00	25.62 O
ATOM	57	CB	PHE	A	319	−4.532	−0.862	28.741	1.00	26.12 C
ATOM	58	CG	PHE	A	319	−3.977	0.004	29.823	1.00	26.52 C
ATOM	59	CD1	PHE	A	319	−4.229	1.368	29.839	1.00	30.64 C
ATOM	60	CE1	PHE	A	319	−3.731	2.174	30.867	1.00	31.78 C
ATOM	61	N	ASN	A	320	−6.726	−0.410	26.564	1.00	23.99 N
ATOM	62	CA	ASN	A	320	−6.956	−0.616	25.155	1.00	24.11 C
ATOM	63	C	ASN	A	320	−5.554	−0.926	24.635	1.00	27.35 C
ATOM	64	O	ASN	A	320	−4.654	−0.066	24.695	1.00	24.65 O
ATOM	65	CB	ASN	A	320	−7.569	0.636	24.508	1.00	23.58 C
ATOM	66	CG	ASN	A	320	−7.625	0.595	23.004	1.00	34.43 C
ATOM	67	ND2	ASN	A	320	−8.716	1.031	22.426	1.00	24.70 N
TER	68		ASN	A	320					
ATOM	69	CB	TYR	A	323	−3.987	3.146	23.786	1.00	24.01 C
ATOM	70	CG	TYR	A	323	−5.324	3.669	23.316	1.00	27.27 C
ATOM	71	CD2	TYR	A	323	−6.338	3.953	24.224	1.00	27.85 C
ATOM	72	CE2	TYR	A	323	−7.551	4.485	23.803	1.00	28.48 C
ATOM	73	cz	TYR	A	323	−7.760	4.733	22.459	1.00	39.89 C
TER	74		TYR	A	323					
ATOM	75	O	SER	A	343	−8.221	1.305	36.401	1.00	35.47 O
ATOM	76	CA	VAL	A	344	−9.112	3.668	37.518	1.00	31.45 C
ATOM	77	C	VAL	A	344	−8.465	5.023	37.143	1.00	35.04 C
ATOM	78	O	VAL	A	344	−9.164	5.988	36.815	1.00	35.77 O
ATOM	79	CB	VAL	A	344	−10.398	3.839	38.375	1.00	35.78 C
ATOM	80	CG1	VAL	A	344	−11.101	2.498	38.569	1.00	35.22 C
TER	81		VAL	A	344					
ATOM	82	CB	SER	A	346	−3.897	8.687	33.543	1.00	33.22 C
ATOM	83	OG	SER	A	346	−3.189	9.493	34.475	1.00	39.59 O
TER	84		SER	A	346					
ATOM	85	C	VAL	A	360	−1.708	8.441	28.606	1.00	31.20 C
ATOM	86	O	VAL	A	360	−2.317	8.921	27.649	1.00	30.76 O
ATOM	87	CB	VAL	A	360	−0.897	10.498	29.926	1.00	31.93 C
ATOM	88	CG1	VAL	A	360	−2.031	10.304	30.938	1.00	32.54 C
ATOM	89	C	VAL	A	361	−4.106	6.199	29.791	1.00	28.46 C
ATOM	90	CG1	VAL	A	361	−3.751	4.254	27.465	1.00	28.23 C
ATOM	91	N	MET	A	362	−5.362	6.501	29.534	1.00	27.43 N
ATOM	92	CA	MET	A	362	−6.410	6.303	30.527	1.00	28.06 C
ATOM	93	C	MET	A	362	−6.834	4.822	30.434	1.00	30.78 C
ATOM	94	O	MET	A	362	−7.011	4.331	29.325	1.00	29.96 O
ATOM	95	CB	MET	A	362	−7.591	7.238	30.220	1.00	30.87 C
ATOM	96	CG	MET	A	362	−8.518	7.477	31.376	1.00	34.33 C
ATOM	97	SD	MET	A	362	−7.736	8.227	32.828	1.00	37.58 s
ATOM	98	CE	MET	A	362	−9.105	8.087	34.005	1.00	34.59 C
ATOM	99	N	PRO	A	363	−6.981	4.091	31.569	1.00	26.83 N
ATOM	100	CA	PRO	A	363	−7.383	2.677	31.502	1.00	25.64 C
ATOM	101	C	PRO	A	363	−8.853	2.456	31.064	1.00	31.65 C
ATOM	102	O	PRO	A	363	−9.600	3.409	30.801	1.00	29.62 O
ATOM	103	CB	PRO	A	363	−7.148	2.203	32.941	1.00	26.32 C
ATOM	104	CG	PRO	A	363	−7.447	3.380	33.754	1.00	30.73 C
ATOM	105	CD	PRO	A	363	−6.797	4.505	32.971	1.00	26.98 C
ATOM	106	N	MET	A	364	−9.251	1.179	30.970	1.00	28.59 N
ATOM	107	CA	MET	A	364	−10.606	0.744	30.632	1.00	28.63 C
ATOM	108	C	MET	A	364	−11.156	0.010	31.848	1.00	33.94 C
ATOM	109	O	MET	A	364	−10.376	−0.602	32.572	1.00	30.49 O
ATOM	110	CB	MET	A	364	−10.559	−0.264	29.473	1.00	30.86 C
ATOM	111	CG	MET	A	364	−10.480	0.379	28.110	1.00	34.27 C
ATOM	112	SD	MET	A	364	−10.177	−0.880	26.856	1.00	37.20 S
ATOM	113	CE	MET	A	364	−11.844	−1.575	26.686	1.00	34.67 C
ATOM	114	N	ARG	A	365	−12.490	0.042	32.084	1.00	36.79 N
ATOM	115	CA	ARG	A	365	−13.039	−0.701	33.225	1.00	38.26 C
ATOM	116	C	ARG	A	365	−13.003	−2.213	32.924	1.00	41.53 C
ATOM	117	O	ARG	A	365	−13.457	−2.647	31.869	1.00	41.37 O
ATOM	118	CB	ARG	A	365	−14.441	−0.209	33.655	1.00	40.76 C
ATOM	119	CG	ARG	A	365	−14.900	−0.881	34.954	1.00	49.70 C
ATOM	120	CD	ARG	A	365	−16.194	−0.321	35.483	1.00	57.33 C
ATOM	121	NE	ARG	A	365	−15.967	0.754	36.451	1.00	62.68 N
ATOM	122	CZ	ARG	A	365	−16.403	1.998	36.291	1.00	83.24 C

TABLE 10-continued

Atomic coordinates of P14 residues and of the residues involved in the binding of P14 to the β clamp, in the crystal of P14 peptide co-crystallized with the β ring.

ATOM	123	NH1	ARG	A	365	−17.085	2.335	35.202	1.00	79.01	N1+
ATOM	124	NH2	ARG	A	365	−16.159	2.916	37.217	1.00	72.47	N
TER	125		ARG	A	365						
HETATM	126	O	HOH	A	393	−12.258	0.479	23.904	1.00	30.49	O
HETATM	127	O	HOH	A	434	−11.324	4.559	22.768	1.00	39.46	O
HETATM	128	O	HOH	A	453	−10.378	2.373	23.903	1.00	29.67	O
HETATM	129	O	HOH	A	463	−8.977	20.736	30.414	1.00	61.87	O
HETATM	130	O	HOH	A	490	−11.833	19.233	26.362	1.00	59.30	O
HETATM	131	O	HOH	A	494	−9.709	19.924	32.759	1.00	46.54	O
HETATM	132	O	HOH	A	516	−16.291	−3.291	31.343	1.00	50.13	O
HETATM	133	O	HOH	C	45	−6.988	2.274	27.402	1.00	24.52	O
HETATM	134	O	HOH	C	219	−14.432	8.577	32.316	1.00	42.19	O
HETATM	135	O	HOH	C	236	−12.880	18.057	33.132	1.00	45.93	O
HETATM	136	C	ACE	C	367	−14.148	1.368	29.378	1.00	30.45	C
HETATM	137	O	ACE	C	367	−13.835	1.713	30.502	1.00	36.32	O
HETATM	138	CH3	ACE	C	367	−15.035	0.134	29.141	1.00	23.39	C
ATOM	139	N	GLN	C	368	−13.746	1.957	28.290	1.00	33.32	N
ATOM	140	CA	GLN	C	368	−12.873	3.100	28.115	1.00	32.69	C
ATOM	141	C	GLN	C	368	−13.283	4.349	28.853	1.00	33.34	C
ATOM	142	O	GLN	C	368	−14.330	4.821	28.622	1.00	32.62	O
ATOM	143	CB	GLN	C	368	−12.617	3.443	26.659	1.00	30.16	C
ATOM	144	CG	GLN	C	368	−11.470	4.502	26.458	1.00	29.48	C
ATOM	145	CD	GLN	C	368	−10.087	4.218	27.096	1.00	29.80	C
ATOM	146	NE2	GLN	C	368	−9.514	3.231	26.666	1.00	25.11	N
ATOM	147	OE1	GLN	C	368	−9.495	4.916	28.005	1.00	31.46	O
TER	148		GLN	C	368						
HETATM	149	N	ALC	C	369	−12.348	4.783	29.710	1.00	32.73	N
HETATM	150	CA	ALC	C	369	−12.500	6.004	30.548	1.00	34.14	C
HETATM	151	C	ALC	C	369	−11.878	7.192	29.752	1.00	36.64	C
HETATM	152	O	ALC	C	369	−10.984	7.069	28.869	1.00	32.46	O
HETATM	153	CB	ALC	C	369	−11.739	5.830	31.889	1.00	34.55	C
HETATM	154	CG	ALC	C	369	−12.265	4.559	32.650	1.00	35.64	C
HETATM	155	CD1	ALC	C	369	−13.808	4.654	32.962	1.00	39.49	C
HETATM	156	CD2	ALC	C	369	−11.323	4.206	33.835	1.00	33.24	C
HETATM	157	CE1	ALC	C	369	−14.214	3.964	34.302	1.00	41.77	C
HETATM	158	CE2	ALC	C	369	−11.911	3.020	34.514	1.00	36.86	C
HETATM	159	CZ	ALC	C	369	−13.067	3.588	35.295	1.00	40.10	C
ATOM	160	N	ASP	C	370	−12.406	8.334	30.145	1.00	40.84	N
ATOM	161	CA	ASP	C	370	−12.054	9.670	29.629	1.00	43.94	C
ATOM	162	C	ASP	C	370	−10.905	10.238	30.494	1.00	41.92	C
ATOM	163	O	ASP	C	370	−10.900	10.094	31.706	1.00	39.99	O
ATOM	164	CB	ASP	C	370	−13.194	10.609	29.791	1.00	48.03	C
ATOM	165	CG	ASP	C	370	−14.398	10.233	29.003	1.00	55.27	C
ATOM	166	OD1	ASP	C	370	−14.373	9.336	28.084	1.00	56.63	O
ATOM	167	OD2	ASP	C	370	−15.398	10.873	29.360	1.00	59.41	O1−
ATOM	168	N	LEU	C	371	−9.929	10.917	29.882	1.00	43.04	N
ATOM	169	CA	LEU	C	371	−8.785	11.787	30.606	1.00	42.42	C
ATOM	170	C	LEU	C	371	−9.335	12.911	31.330	1.00	47.23	C
ATOM	171	O	LEU	C	371	−8.808	13.271	32.377	1.00	47.55	O
ATOM	172	CB	LEU	C	371	−7.621	12.265	29.726	1.00	37.30	C
ATOM	173	CG	LEU	C	371	−6.599	11.206	29.251	1.00	32.23	C
ATOM	174	CD1	LEU	C	371	−5.841	11.622	27.996	1.00	27.72	C
ATOM	175	CD2	LEU	C	371	−5.712	10.688	30.370	1.00	32.57	C
TER	176		LEU	C	371						
HETATM	177	N	ZCL	C	372	−10.331	13.589	30.796	1.00	51.48	N
HETATM	178	CA	ZCL	C	372	−11.007	14.833	31.271	1.00	56.21	C
HETATM	179	C	ZCL	C	372	−12.356	14.343	31.982	1.00	59.74	C
HETATM	180	O	ZCL	C	372	−13.391	14.104	31.274	1.00	58.69	O
HETATM	181	CB	ZCL	C	372	−11.091	16.085	30.288	1.00	55.89	C
HETATM	182	CG	ZCL	C	372	−9.672	16.291	29.799	1.00	57.05	C
HETATM	183	CD1	ZCL	C	372	−9.347	15.682	28.574	1.00	57.40	C
HETATM	184	CD2	ZCL	C	372	−8.663	17.020	30.494	1.00	57.44	C
HETATM	185	CE1	ZCL	C	372	−8.088	15.803	28.089	1.00	59.91	C
HETATM	186	CE2	ZCL	C	372	−7.347	17.172	29.984	1.00	57.91	C
HETATM	187	CZ	ZCL	C	372	−7.057	16.515	28.746	1.00	57.04	C
HETATM	188	CLE1	ZCL	C	372	−7.803	15.045	26.635	1.00	66.69	CL
HETATM	189	CLZ	ZCL	C	372	−5.507	16.525	27.966	1.00	50.27	CL
HETATM	190	OXT	ZCL	C	372	−12.339	14.160	33.254	1.00	61.54	O
END											

1.2.5. Thermodynamic Analysis of the Pseudo Peptides Interaction with the Ring.

ITC experiments were conducted on selected peptides in order to determine the thermodynamics parameters of their interaction with the ring (Table 11 and FIG. **8**). Although the K_d values determined in these experimental conditions were slightly different from those obtained by SPR, the same tendency was observed for all peptides (Table 11). The largest differences are observed for low affinity peptides (P1, P3, P6) while, for higher affinity peptides (P7, P11, P12, P13 and

P14), both techniques yielded similar values. The introduction of modifications, Cha group in position 2 and p-methyl, p-chloro and p-bromo groups on F_5, increases the affinity of the ligand, reaching respectively about 380, 100, 65 and 150 nM range, as compared to the 1-2 μM affinity of the reference natural peptide P1 (Table 11). A strong correlation is observed between ΔH and ΔS values (FIG. **8**), which reflects an enthalpy-entropy compensatory effect, already observed in other systems[21, 22]. This correlation accounts for the small ΔG variation among the various peptides (FIG. **8**). As reported earlier[23], this correlation suggests that the observed desolvation of the pocket upon ligand interaction plays a major role in the binding process.

TABLE 11

ITC experiments on selected β binding peptides.

Beta (μM)	Pep (μM)	Name	N	Kd (nM)	IC_{50} SPR (nM)	ΔH (cal/Mol)	ΔS (cal/ mol/deg)	ΔG (Kcal/mol)
30	400	P1 (SEQ ID No: 1)	1.33	1579	8850	-4087	12.8	-7.9
		RQLVLGL	1.31	2012		-5500	7.6	-7.7
30	400	P3 (SEQ ID No: 3)	1.54	2816	8620	-5769	6.05	-7.5
		RQLVLF	1.45	2320		-5699	6.6	-7.6
30	400	P6 (SEQ ID No: 6)	1.11	820	1120	$-1.13\ 10^4$	-10.4	-8.1
		AcQLDLF	1.14	613		$-1.11\ 10^4$	-9.08	-8.3
30	400	P7 (SEQ ID No: 7)	0.74	246	170	$-2.13\ 10^4$	-41.2	-8.9
		AcQChaDLF	0.76	222		$-2.05\ 10^4$	-38.5	-8.9
20	400	P11 (SEQ ID No: 11)	0.95	401	260	$-1.48\ 10^4$	-20.4	-8.6
		AcQChaDLpMeF	0.95	362		$-1.44\ 10^4$	-19.0	-8.6
20	400	P12 (SEQ ID No: 12)	1.02	89	160	$-1.5\ 10^4$	-18.1	-9.6
		AcQChaDLpClF	1.06	122		$-1.43\ 10^4$	-16.3	-9.4
20	400	P13 (SEQ ID No: 13	0.85	136	96	$-1.81\ 10^4$	-29.3	-9.3
		AcQChaDLpBrF	0.83	167		$-1.52\ 10^4$	-20.2	-9.1
20	400	P14 (SEQ ID No: 14)	0.91	73	77	$-1.89\ 10^4$	-30.8	-9.6
		AcQChaDLdiClF	0.90	55		$-1.81\ 10^4$	-27.6	-9.8

ΔG = ΔH-TΔS.

All experiments were performed at 25° C. Results from SPR experiments (IC50) are added for comparison purposes.

N: number of binding sites per β monomer.

The effect of the various modifications introduced in the peptides can be estimated from the ΔΔG values (see Table 12). The Cha moiety in position 2 contributes to the increased interaction by −0.66 kcal/mol as compared to the natural pentapeptide P6. While the introduction of a methyl substitution on the ring of the terminal phenylalanine residue is detrimental to the affinity (+0.25 kcal/mol), halogen modification results in a graduate effect on ligand affinity (p-bromo<p-chloro<3,4-dichloro). The chloro modification in para position contributes for the main part to the increased affinity (−0.6 kcal/mol). This contribution can result partly from an increased hydrophobic character of the halogen modified F residue[24], as well as from dehydration of both peptide and sub site 1[25] and reorganization of water molecules as observed by comparing free and bound β interacting pockets (FIG. **1**AB). In comparison, the second chlorine atom (meta position) in P14 only contributes for −0.2 kcal/mol and the para-bromo modification contribution to the binding energy is two times lower (−0.28 kcal/mol) than the corresponding para-chloro modification.

TABLE 12

effects of modifications introduced in the various pentapeptides measured from the ΔΔG values extracted from ITC experiments data. The P6 sequence (AcQLDLF) is chosen as a reference. ΔΔG = ΔGj − ΔGi.

	ΔGi/ΔGj										
	P6/ P7	P7/ P11	P7/ P12	P7/ P14	P7/ P13	P11/ P12	P11/ P14	P11/ P13	P12/ P14	P13/ P12	P13/ P14
Compared residues	L/ Cha	F/ pMeF	F/ pClF	F/ diClF	F: pBrF	pMeF/ pClF	pMeF/diClF	pMeF/ pBrF	pClF/ diClF	pBrF/ pClF	pBrF/ diClF
ΔΔG (Kcal/mol)	−0.66	+0.25	−0.6	−0.8	−0.28	−0.85	−1.05	−0.53	−0.2	−0.32	−0.52

1.3. Discussion.

1.3.1. The Fully Efficient β Binding Pocket is Formed Upon Ligand Binding.

Many cellular factors involved in replication and genome integrity survey processes require a peptide mediated interaction with the replisome sliding clamp in order to fulfill their function. This interaction has been structurally fully characterized in prokaryotes[16, 17, 18] and eucaryotes[2 26]. In our previously published structure (1OK7), the β ring interacts with only one peptide, leaving one binding pocket free[17]. This gives the opportunity to compare the structure of a peptide-free versus a peptide-bound pocket and to get insights into the dynamic of the pocket upon peptide binding. Although the general structures of the free or bound pockets are similar, as estimated by the Cα chain conformation (rmsd=0.36 Å), the side chains of several residues undergo major movements (FIG. **6**), notably residues M_{362} and S_{346} side chains which are displaced in a concerted way, and residue R_{365}. The concerted shift of M_{362} and S_{346} side chains probably is a structural marker for the presence of a peptide ligand in the binding pocket. In absence of the ligand, these residues adopt a so-called close conformation where the M_{362} side chain is oriented toward residue H_{175} and separates subsite 1 and subsite 2 (PDB IDs 2POL, 1MMI and 1OK7 (monomer A)) (FIG. **6**). Alternatively, in all β ring bound structures (1UNN, 1OK7 (monomer B), 3D1E, 3D1F), the residues are shifted by 180° in an open conformation, allowing the opening of a cleft joining the two subsites (FIG. **1**BC). One exception is found in the structure of β co-crystallized with the Pol II peptide (3D1E) where no ligand is observed in monomer B, although the two residues adopt an open conformation[18]. This may result from a partial occupancy of the pocket, making difficult the detection of the peptide.

Residue R_{365} is also shifted by an angle of 46° toward residue L_{366}, triggering the opening of a platform shaped by R_{365}, P_{363}, M_{362} and V_{344}, where the L_3 residue of the peptide locates (FIG. **1**BC). The global dynamic of this structural modification has been modeled, showing that, as the ligand binds into the pocket, a groove forms that joins subsite 1 and subsite 2, in which the extended peptide can adapt.

The correlation between side chains orientations and the presence of a peptide in the binding pocket suggests that these two side chains might play a strategic function in the ligand binding process. The pocket could adopt two configurations: a closed configuration where the M_{362} side chains lies in the path between the two subsites of the pocket, thus impeding the formation of an efficient binding site (FIG. **1**A). Alternatively, an open configuration where the M_{362} side chains shifts by about 180°, allowing ligand binding into the groove that joins the two subsites, as well as the opening of the platform so that the peptide establishes optimal interactions. At present, it is not known if the fully efficient binding pocket is readily available at the surface of the protein, or is structured by the binding of a specific ligand, according to a bona fide induced fit model. Previous observations for a ligand binding site on the cytokine IL-2[27] reveal that a portion of the binding site is adaptive and can form a hydrophobic channel upon ligand binding. A similar adaptive process could occur for the β binding pocket and would ensure the binding specificity of ligand proteins. Although the details of the dynamic process of this binding site formation are not known yet, this observation is likely to have major implications for the design of high affinity ligands.

1.3.2. Structure-Based Design of High Affinity Ligands.

The inventors have studied the interaction of various peptides with the *E. coli* processivity ring. Starting from the natural sequence of the Pol IV DNA polymerase interacting peptide (P1, RQLVLGL, SEQ ID No: 1), they have sequentially modified the peptide in order to increase its interaction strength and concomitantly to limit its size. The affinities were measured by biochemical assays, SPR and ITC, and despite a difference in sensitivity of these various techniques, the relative affinities of the peptides were similar. From the P1-β complex (PDB 1OK7), a minimal acetylated peptide (P6, AcQLDLF) was first delineated and found to bind about eight times tighter than P1. Then, the crystallographic structure of the P1-β and P6-β complexes were used together with modelling informations to guide modifications at specific positions (corresponding to residues L_2 and F_5 of P6, AcQLDLF), resulting in a dramatic increase of the modified peptides affinity for the β ring interacting pocket. This gain essentially results from an increase in hydrophobic interactions. A first improvement was achieved by the introduction of a cyclohexylalanyl residue at the P6-L2 position. Although

position 2 does not correspond to a major binding pocket on the β-clamp, the simple modification to a cyclohexylalanyl residue (P7) improved the affinity by a factor 6 with respect to the P6 peptide (Table 1), indicating that shallow secondary sites can still prove useful in a global optimization scheme.

The other interesting gain was achieved by the addition of halogen atoms on the benzyl ring of the F residue. Interestingly, the chlorine atom when introduced at the meta position, forms a halogen bond that further strengthens the interaction. P14, which combines both the Cha and a 3,4-dichloro phenylalanine residue, displays the highest affinity, around 60 (±10) nM as measured by ITC, which represents a 10 to 15 fold increase in binding as compared to P6 and a 4 fold increased as compared to the whole PolIV DNA polymerase. The double F ring substitution contributes for −0.8 kcal/mol to the overall interaction. The para-chloro substitution provides most of the contribution to the binding while the meta-chloro, involved in a halogen bond, only contributes for one fourth of the total free energy.

Interestingly, halogen substituted aromatic ligands were also recently independently identified for the β-clamp using a chemical library screening strategy. A compound, named RU67, was identified as a β ligand inhibiting the *E. coli* pol III enzyme with a Ki of 10 μM[18]. It contains a di-bromo substituted aromatic ring which is deeply inserted in sub site 1 and almost superimposes with the peptide L_4 residue of the Pol IV binding peptide. One bromine atom does not form any interaction while the other forms a halogen bond with β residue T_{172} (d=3.02 Å and θ=133.21°). This latter interaction is similar to that observed for the chlorine atom in meta position of F_5 in P14.

This specific positioning of halogen substituted ligands in subsite 1 of the β-clamp contrasts with the wider range of positions occupied by natural amino acids in the same pocket. Indeed, the comparison of different structures of β in complex with various natural peptides (1OK7, 3D1E, 3D1F)[17, 18] reveals that, while most of the peptide residues adopt the same overall conformation within the pocket, the position of the last C-terminal residues of the interacting sequence varies in subsite 1. This suggests that no specific interaction is established with specific β residue, but instead that this hydrophobic interaction, delocalized in all subsite 1, contributes mainly to paste the peptide onto the β surface.

In conclusion, the structure-based approach described herein allows the design of ligands that bind two orders of magnitude tighter than the natural peptide P1, reaching the 10^{-8} M range, and 4 fold more than the Pol IV enzyme. This increase in affinity relies both on chemical substitutions introduced on the peptide that increase the hydrophobic interactions and on the fact that the bidentate interaction of the ligand in the binding pocket is retained. This interaction mode promotes the modeling of an efficient binding site, possibly through an induced-fit process.

Further designs of high affinity ligands should also take into account the dynamic nature of the binding site formation. These observations are likely to have major implications for the development of new antibiotic compounds.

Example 2

Synthesis and Functional Characterization of Further Peptides Derived from P7 (SEQ ID No: 7), P12 (SEQ ID No: 12), P14 (SEQ ID No: 14), P23-P30 (SEQ ID Nos: 23-30)

Compounds P23 to P28 (SEQ ID No: 23 to SEQ ID No: 28), which are analogues of P7 and P14, have been prepared to (i) remove carboxylic side functions and monitor the influence of R and guanidino groups and conformational constraint. Compounds P23 to P28 have been prepared as previously reported in example 1 starting from Fmoc-Phe Wang resin or from o-chlorotrityl chloride resin. The linear precursor of P29 (Ac-RQChaKLF-OH, P27, SEQ ID No: 27) is prepared as described in example 1. In the case of compound P30 (SEQ ID No: 30), the linear precursor P28 (SEQ ID No: 28) requires the use of a beta amino acid (Fmoc-beta-HPhe-OH) which is commercially available from Sigma-Aldrich. In the case of P29 and P30, lactam formation between the amino group of the lysine side chain and the C-terminal carboxylic function was performed using standard methods as described in the literature (Robert C. Reid, Giovanni Abbenante, Stephen M. Taylor, and David P. Fairlie; J. Org. Chem., 2003, 68 (11), pp 4464-4471). The cyclization which requires the activation and coupling of a peptidyl-Arg residue (P27 or P28) was carried out in DMF at room temperature using BOP as a coupling agent and diisopropylethylamine as the base and monitored by analytical

RP-HPLC. Briefly, a solution of the fully deprotected peptide 27 or 28 (1 equivalent) in DMF (10-1M) and diisopropylethylamine (5.5 equivalent) was stirred at room temperature (about 20° C.) until homogeneous. Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate or BOP reagent (1.08 equivalent) was added, and the solution was stirred at room temperature (about 20° C.) for 2 h. A saturated Bicarbonate solution was added and the solid product was filtered off, washed on the filter with ether, and dried under high vacuum. The crude product was dissolved in 50% CH_3CN/50% water (1 L) and purified by C18 RP-HPLC. The peak containing the cyclic peptide was collected and lyophilized.

Example 3

New Peptides Derived from P14, SPR Experiments

Based on the crystals analysis, the inventors have used peptide P14 (Bu29) as a scaffold to derive new variants with the aim of deciphering new properties to the ligand such as increased affinity, increased solubility, or increased cell penetration.

TABLE 13

sequences of the P14 derivative peptides.

Peptide name	Sequence	Mw	SEQ ID No:
P14	Ac Q Cha D L diClF		14
P14-4	Ac R Q Cha N L diClF	940	36
P14-5	Cin R Q Cha N L diClF	1028	37
P14-6	Ac R Q Cha R L diClF	980.5	38
P14-7	Ac R Q Cha R L F	913	39
P14-8	Ac R Q Cha K L F	886	40

Ac: acetyl group;
Cin: cinnamoyl group.

The rationale directing the design of these peptides was 1) to extend the interaction of the N-terminal part of the peptide with the edge of the binding pocket, 2) to take advantage of the position of the D residue of peptide P14, that points

towards the solvent, to introduce positively charged residues that will increase the solubility and the membrane penetrating capabilities of the peptide.

The affinity of these peptides for the binding pocket was measured by SPR, by defining the concentration of ligand that challenges 50% of the binding of the natural peptide P1 (RQLVLGL), thus defining a Ki. Results are given in table 14

TABLE 14

relative affinity of P14 derivative peptides for the beta ring binding pocket, as measured by SPR experiments.

Peptide name	Sequence	Ki (nM)	SEQ ID No:
P7	Ac Q Cha D L F	170	7
P14	Ac Q Cha D L diClF	85	14
P14-4	Ac R Q Cha N L diClF	insoluble	36
P14-5	Cin R Q Cha N L diClF	3250	37
P14-6	Ac R Q Cha R L diClF	>10 000	38
P14-7	Ac R Q Cha R L F	>10 000	39
P14-8	Ac R Q Cha K L F	904	40

CONCLUSIONS

None of the peptides is a better binder than P14. One of them, P14-8, still bind the target with a 1 μM affinity.

As compared to P14 (SEQ ID No: 14), the introduction of R and K residues in the sequence (P14-8, SEQ ID No: 40) results in a 10 fold decrease in affinity.

The diClF to F substitution results in a 2 fold decrease in affinity (compare P14 and P7). Thus the R and K subtitutions in peptide P14-8 may contribute to a 5 fold decrease (1000/200) of the affinity as compared to P14.

P14-7 and P14-8 differ from each other by the 5^{th} residue (R for #7 and K for #8). Comparison of the relative affinity of these two peptides suggests that K residue is a better choice to maintain affinity. Moreover, comparing P7 and P14-7, which differ by R1 and R5 residues, indicates an affinity ratio of at least 50 (#10 000/200).

Altogether, this suggests that the K5 residue in P14-8 may contribute to decrease the affinity by a factor of 10 (P14-7/P7=5 and P14-8/P7=50).

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Arg Gln Leu Val Leu Leu
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Arg Gln Leu Val Leu Phe
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Arg Gln Leu Val Phe Leu
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Gln Leu Asp Leu Phe
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Gln Leu Asp Leu Phe
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Gln Xaa Asp Leu Phe
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Gln Xaa Asp Leu Phe
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<400> SEQUENCE: 9

Gln Xaa Asp Leu Phe
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Gln Xaa Asp Leu Phe
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Gln Xaa Asp Leu Xaa
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Gln Xaa Asp Leu Xaa
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Gln Xaa Asp Leu Xaa
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Gln Xaa Asp Leu Xaa
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Arg Gln Leu Val Leu Phe
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Gln Xaa Asp Xaa Phe
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Gln Xaa Asp Xaa Phe
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Gln Xaa Asp Xaa Phe
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Gln Xaa Asp Xaa Phe
1 5

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Gln Xaa Asp Leu Xaa
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<400> SEQUENCE: 21

Gln Xaa Asp Leu Trp
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Gln Xaa Asp Leu Xaa
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<223> OTHER INFORMATION: Xaa = di-chloro-phenylalanine carboxamide

<400> SEQUENCE: 23

Gln Xaa Asn Leu Xaa
1 5

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<400> SEQUENCE: 24

Arg Gln Xaa Asn Leu Xaa
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<223> OTHER INFORMATION: Xaa = di-chloro-phenylalanine carboxamide

<400> SEQUENCE: 25

Arg Gln Xaa Arg Leu Xaa
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<223> OTHER INFORMATION: Xaa = di-chloro-phenylalanine carboxamide

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<400> SEQUENCE: 26

Gln Xaa Arg Leu Xaa
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<400> SEQUENCE: 27

Arg Gln Xaa Lys Leu Phe
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<220> FEATURE:
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<223> OTHER INFORMATION: Xaa = beta homophenylalanine

<400> SEQUENCE: 28

Arg Gln Xaa Lys Leu Xaa
1 5

<210> SEQ ID NO 29
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<223> OTHER INFORMATION: The peptide is cyclized between residues K
and F

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Arg Gln Xaa Lys Leu Phe
1 5

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Arg Gln Xaa Lys Leu Xaa
1 5

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Ala Ser Arg Gln
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Arg Gln Leu Val Leu Gly Leu
1 5

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Xaa Phe Gln Leu Asp
1 5

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Arg Gln Xaa Asn Leu Xaa
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Arg Gln Xaa Asn Leu Xaa
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Arg Gln Xaa Arg Leu Xaa
1 5

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<223> OTHER INFORMATION: Xaa = Cha

<400> SEQUENCE: 39

Arg Gln Xaa Arg Leu Phe
1 5

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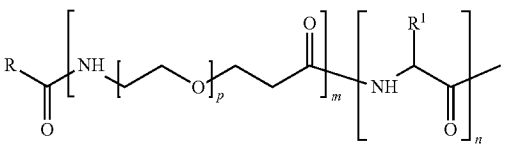
Arg Gln Xaa Lys Leu Phe
1 5

The invention claimed is:

1. A compound of formula (I)



(I)

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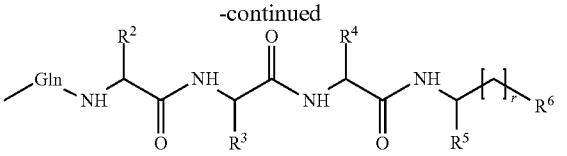

wherein

Gln is glutamine;

R is selected from the group consisting of a C_{1-12}-alkyl group optionally substituted by a C_{6-10}-aryl group, a C_{2-12}-alkenyl group optionally substituted by a C_{6-10}-aryl group, a C_{3-6}-cycloalkyl group, a C_{6-10}-aryl group

optionally substituted by a C_{1-4}-alkyl, and a C_{1-5}-alkyl-(O—CH_2—CH_2)$_t$— group with t being an integer from 0 to 20 inclusive;

R^1 is the side chain of arginine or lysine;

R^2 is a —(CH_2)—C_{3-6}-cycloalkyl group optionally substituted by a halogen and/or by a group selected from the group consisting of —NH_2, —NH—CO—R^a, —CO_2H, —NHR^a and —NR^aR^b, wherein R^a and R^b are independently a C_{1-4}-alkyl group;

R^3 is selected from the group consisting of a C_{1-8}-alkyl group, the side chain of arginine or lysine, —(CH_2)$_q$—CO_2R^{7a}, —(CH_2)$_q$—CO—NHR^{7b}, —CH_2OR^8 and —(CH_2)$_q$$NHR^9$, wherein

q is 1, 2, 3 or 4,

R^{7a} is a hydrogen atom, a C_{1-8}-alkyl group, a C_{4-12}-alkylene group forming together with R^6 a lactone or a polyether ring, or a C_{4-12}-alkenylene, forming together with R^6 a lactone or a polyether ring,

R^{7b} is a hydrogen atom, a C_{1-8}-alkyl group, or —(CH_2)$_{q'}$—NH— with q' being an integer between 2 and 8 inclusive and forming together with R^6 a lactam,

R^8 is a hydrogen atom, a C_{1-8}-alkyl group, a C_{4-12}-alkylene group forming together with R^6 a lactone or a polyether ring, or a C_{4-12}-alkenylene, forming together with R^6 a lactone or a polyether ring,

R^9 is a hydrogen atom, or R^9 together with R^6 form a lactam;

R^4 is a C_{1-8}-alkyl group optionally substituted by a C_{3-6}-cycloalkyl group, or a halogen-C_{1-4}-alkyl group;

R^5 is selected from the group consisting of a —(CH_2)—C_{3-6}-cycloalkyl group; —(CH_2—CH_2)—C_{3-6}-cycloalkyl group; a —(CH_2)—C_{6-10}-aryl group optionally substituted by a halogen, a C_{1-2} alkyl group and/or a C_{1-2} alkoxy group; a —(CH_2—CH_2)—C_{6-10}-aryl group optionally substituted by a halogen, a C_{1-2} alkyl group and/or a C_{1-2} alkoxy group; a —(CH_2)—C_{6-10}-heteroaryl group optionally substituted by a halogen and/or a C_{1-2} alkyl group; and a —(CH_2—CH_2)—C_{5-10}-heteroaryl group optionally substituted by a halogen and/or a C_{1-2} alkyl group;

R^6 is —CO_2H, —CO_2R^{10}, —CO—NH_2, —CO—NHR^{10}, —OR^{10} when r is 1 or 2, —NH—CO—NHR^{10} when r is 1 or 2, or R^6 is —CO—, —CO—O— or —O— and forms a lactam, a lactone, or a polyether ring with R^{7a}, R^{7b}, R^8 or R^9; wherein

R^{10} is a C_{1-8}-alkyl group optionally substituted by a C_{6-10}-aryl group; a C_{3-6}-cycloalkyl group; a C_{6-10}-aryl group optionally substituted by a halogen, a C_{1-2}-alkyl group and/or a C_{1-2}-alkoxy group;

m is 0 or 1;

n is an integer from 0 to 9 inclusive;

p is an integer from 0 to 10 inclusive;

r is 0, 1 or 2.

2. The compound of claim **1**, wherein

R is a C_{1-8}-alkyl group optionally substituted by a C_{6-10}-aryl group, a C_{2-8}-alkenyl group optionally substituted by a C_{6-10}-aryl group or a C_{1-6}-alkyl-(O—CH_2—CH_2)$_t$ — group with t being an integer from 0 to 10 inclusive.

3. The compound of claim **1**, wherein

R is a C_{1-4}-alkyl group optionally substituted by a C_{6-10}-aryl group or a C_{2-4}-alkenyl group optionally substituted by a C_{6-10}-aryl group.

4. The compound of claim **1**, wherein n is an integer comprised between 1 and 5 inclusive.

5. The compound of claim **1**, wherein n is 0.

6. The compound of claim **1**, wherein

R^2 is a —(CH_2)—C_{3-6}-cycloalkyl group.

7. The compound of claim **1**, wherein

R^4 is a C_{1-5}-alkyl group or a C_{1-2}-alkyl group optionally substituted by a C_{3-6}-cycloalkyl group.

8. The compound of claim **1**, wherein

R^5 is a —(CH_2)—C_{6-10}-aryl group optionally substituted by a halogen, a C_{1-2} alkyl group and/or a C_{1-2} alkoxy group.

9. The compound of claim **1**, wherein

R^3 is selected from the group consisting of a C_{1-8}-alkyl group, the side chain of arginine or lysine, —(CH_2)$_q$—CO_2R^{7a}, —(CH_2)$_q$—CO—NHR^{7b}, —CH_2OR^8, and —(CH_2)$_q$$NHR^9$, wherein

q is 1, 2, 3, 4,

R^{7a} is a hydrogen atom, or a C_{1-8}-alkyl group,

R^{7b} is a hydrogen atom, or a C_{1-8}-alkyl group,

R^8 is a hydrogen atom, a C_{1-8}-alkyl group,

R^9 is a hydrogen atom;

and

R^6 is —CO_2H, —CO_2R^{10}, —CO—NH_2, —CO—NHR^{10}, —OR^{10} when r is 1 or 2, —NH—CO—NHR^{10} when r is 1 or 2; wherein

R^{10} is a C_{1-8}-alkyl group optionally substituted by a C_{6-10}-aryl group; a C_{3-6}-cycloalkyl group; a C_{6-10}-aryl group optionally substituted by a halogen, a C_{1-2}-alkyl group and/or a C_{1-2}-alkoxy group.

10. The compound of claim **1**, wherein

R^3 is selected from the group consisting of the side chain of arginine, the side chain of lysine, —(CH_2)$_q$—CO_2R^{7a} and —(CH_2)$_q$—CO—NHR^{7b}, wherein

q is 1, 2, 3 or 4,

R^{7a} is a hydrogen atom, or a C_{1-8}-alkyl group, and

R^{7b} is a hydrogen atom, or a C_{1-8}-alkyl group.

11. The compound of claim **1**, wherein

R^6 is —CO_2H or —CO—NH_2.

12. The compound of claim **1**, wherein

R^3 is selected from the group consisting of —(CH_2)$_q$—CO_2R^{7a}, —(CH_2)$_q$—CO—NHR^{7b}, —CH_2OR^8, and —(CH_2)$_q$$NHR^9$, wherein

q is 1, 2, 3 or 4,

R^{7a} is a C_{4-8}-alkylene group forming together with R^6 a lactone or a polyether ring, or a C_{4-8}-alkenylene, forming together with R^6 a lactone or a polyether ring,

R^{7b} is —(CH_2)$_{q'}$—NH— with q' being an integer from 2 to 8 inclusive and forming together with R^6 a lactam,

R^8 is a C_{4-8}-alkylene group forming together with R^6 a lactone or a polyether ring, or a C_{4-8}-alkenylene, forming together with R^6 a lactone or a polyether ring,

R^9 together with R^6 form a lactam;

R^6 is —CO—, —CO—O— or —O— and forms a lactam, a lactone, or a polyether ring with R^{7a}, R^{7b}, R^8 or R^9.

13. The compound of claim **1**, which is selected from the group consisting of Ac-Gln-Cha-Asp-Leu-Phe (SEQ ID NO:7), Ac-Gln-Cha-Asp-Leu-pMePhe (SEQ ID NO:11), Ac-Gln-Cha-Asp-Leu-pClPhe (SEQ ID NO:12), Ac-Gln-Cha-Asp-Leu-pBrPhe (SEQ ID NO:13), Ac-Gln-Cha-Asp-Leu-diClPhe (SEQ ID NO:14), Ac-Gln-Cha-Asp-hLeu-Phe (SEQ ID NO:16), and Ac-Gln-Cha-Asp-Cha-Phe (SEQ ID NO:17).

14. The compound of claim **1**, wherein the affinity of said compound for the interacting pocket of the bacterial β ring is

at least twice the affinity of the acetylated peptide of sequence AcQLDLF (SEQ ID NO:6) with said interacting pocket.

15. A pharmaceutical composition comprising, as an active agent, a compound according to claim **1**.

16. A method of treating an infection by *E. coli* in a subject in need thereof comprising administering the compound of claim **1** to the subject.

* * * * *